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Science of the Total Environment

USE OF A WASTEWATER RECOVERY PRODUCT (STRUVITE) TO ENHANCE SUBTROPICAL SEAGRASS RESTORATION

--Manuscript Draft--

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Abstract:	Seagrasses are in decline worldwide, and their restoration is relatively expensive and unsuccessful compared to other coastal systems. Fertilization can improve seagrass growth in restoration but can also release nutrients and pollute the surrounding ecosystem. A slow-release fertilizer may reduce excessive nutrient discharge while still providing resources to the seagrass's rhizosphere. In this study, struvite (magnesium ammonium phosphate), a relatively insoluble, sustainable compound harvested in wastewater treatment plants, was compared to OsmocoteTM (14:14:14 Nitrogen: Phosphorus: Potassium, N:P:K), a popular polymer coated controlled release fertilizer commonly used in seagrass restoration. Two experiments compared the effectiveness of both fertilizers in a subtropical flow-through mesocosm setup. In the first experiment, single 0.5 mg of P per g dry weight (DW) doses of OsmocoteTM and struvite fertilizers were added to seagrass plots. Seagrass shoot counts were significantly higher in plots fertilized with struvite than both the OsmocoteTM and unfertilized controls ($p < 0.0001$). A significant difference in total P concentrations was observed in porewater samples of OsmocoteTM vs struvite and controls ($p < 0.0001$), with struvite fertilized plots emitting more than controls ($p < 0.0001$), but less than 2% of the total dissolved P (TDP) of OsmocoteTM fertilized plots (100+ mg/L versus $x > 5$ mg/L). A subsequent experiment, using smaller doses (0.01 and 0.025 mg of P per gram DW added), also found that the struvite treatments performed better than OsmocoteTM, with 16-114% more aboveground biomass (10-60% higher total biomass) while releasing less N and P. These results indicate the relatively rapid dissolution of OsmocoteTM may pose problems to restoration efforts, especially in concentrated doses and possibly leading to seagrass stress. In contrast, struvite may function as a slow-release fertilizer applicable in seagrass and other coastal restoration efforts.
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Submission date: 1/11/22

Professor Damià Barceló
Co-Editor in Chief
Science of the Total Environment

Dear Professor Damià Barceló,

We are pleased to submit our manuscript entitled: "Use of wastewater recovery product (struvite) to enhance subtropical seagrass restoration", for consideration as an original research paper. This study consists of two comparative mesocosm experiments testing the effectiveness of *in situ* mineral fertilization on seagrass restoration. Seagrass is one of the most ecologically valuable marine ecosystems, providing estimated \$29,000 ha^{-1} yr^{-1} in ecosystems services. Yet, despite its value, seagrass beds are declining worldwide in alarming rate.
In situ fertilizer application is a commonly applied seagrass restoration technique, characterized with high rates of success. However, the key drawback of the process is the use of unsustainable fertilizers such as phosphate rock or commercially manufactured controlled release NPK fertilizers. Such an approach can largely offset potential ecological benefits of successful restoration projects. Therefore, we propose the use of a wastewater recovery product, struvite, as an alternative to manufactured commercial fertilizers. Struvite is a widely recognized and applied slow release fertilizer obtained during the wastewater treatment process. Use of struvite introduces elements of sustainability and circular economy in seagrass restoration efforts. In our study, we compared the effects of a popular polymer coated fertilizer (Osmocote) commonly used in seagrass restoration with struvite on seagrass growth and porewater nutrient release. The experiments found that struvite at equivalent doses to Osmocote produced significantly higher seagrass metrics (shoot count and biomass) while emitting significantly fewer nutrients (total dissolved nitrogen and phosphorus). These results demonstrate the potential effectiveness of struvite in seagrass restoration and the relatively rapid dissolution of Osmocote, a potential issue for restoration efforts/local water quality. These findings may be important to providing an effective but low nutrient emission fertilizer for applications in coastal restoration. Within the aims and scope of the Science of the Total Environment, the results of this study are relevant to the subjects of stress ecology in marine ecosystems (or attempts to reduce stress in a sensitive ecosystem) and water quality. This study applies a novel compound in a semi-controlled environment that interconnects with multiple spheres (including the hydrosphere, biosphere, and lithosphere).

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed media. Thank you for your consideration.

Sincerely,

Dr. Conor MacDonnell

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USE OF A WASTEWATER RECOVERY PRODUCT (STRUVITE) TO ENHANCE SUBTROPICAL SEAGRASS RESTORATION

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Response to Reviewers

Reviewer 1

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Other edits

Title Page

T. Osborne changed to T.Z. Osborne.

P. Inglett added as corresponding author.

Manuscript:

Figure 2: Adjusted color scheme of 2B to match 2A.

Tables: Moved all tables to supplementary material, added two tables for sediment nutrients/significant differences.

Mesocosm conditions: Moved from the results section to methods (site description) for greater clarity of environmental conditions.

Abstract

Seagrasses are in decline worldwide, and their restoration is relatively expensive and unsuccessful compared to other coastal systems. Fertilization can improve seagrass growth in restoration but can also ~~excessively ~~release nutrients and pollute the surrounding ecosystem. A slow~~-~~-release fertilizer may reduce excessive nutrient discharge while still providing resources to the seagrass's rhizosphere. In this study, struvite (magnesium ammonium phosphate), a relatively insoluble~~ fertilizer sustainably~~, sustainable compound harvested in wastewater treatment plants, was compared to Osmocote^{TM} (14:14:14 ~~NPK~~Nitrogen: Phosphorus: Potassium, N:P:K), a popular polymer coated controlled release fertilizer commonly used in seagrass restoration. Two experiments compared the effectiveness of both fertilizers in a subtropical flow-through mesocosm setup. In the first experiment, single 0.5 mg of P per g dry weight (DW) doses of Osmocote^{TM} and struvite fertilizers were added to seagrass plots~~ inside a six meter diameter flow-through mesocosm~~. Seagrass shoot counts were significantly higher in plots fertilized with struvite than both the Osmocote^{TM} and unfertilized controls ($p < 0.0001$). A significant difference in total ~~phosphorus~~P concentrations was observed in porewater samples of Osmocote^{TM} vs struvite and controls ($p < 0.0001$), with struvite fertilized plots emitting more than controls ($p \leq 0.0001$), but less than 2% of the total dissolved P (TDP) of Osmocote^{TM} fertilized plots (100+ mg/L versus x > 5 mg/L).~~-~~ A subsequent experiment, using smaller doses (0.01 and 0.025 mg of P per gram DW added), also found that the struvite treatments performed better than Osmocote^{TM}, with 16-114% more aboveground biomass (10-60% higher total biomass) while releasing less ~~nitrogen~~N and ~~phosphorus~~P. These results indicate the relatively rapid dissolution

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Keywords: *Halodule wrightii*; seagrass; marine restoration; fertilizer; struvite; Osmocote^{TM}; phosphorus

1. ~~1.~~ Introduction

Formatted: Outline numbered + Level: 1 + Numbering Style: 1, 2, 3, … + Start at: 1 + Alignment: Left + Aligned at: 0" + Indent at: 0.25"

In many environments, restoration ~~can be~~is improved by fertilization, lessening nutrient limitations and improving growth of desired species (Armitage et al., 2011; Balestri & Lardicci, 2014; Fereidooni et al., 2013; Holmes, 2001; Jaquetti et al., 2014; Reed et al., 2007). ~~For example, Jaquetti et al. (2014) found that fertilization more than doubled the absolute growth rate and significantly improved the photosynthetic response of trees applied in a degraded rainforest restoration site.~~ However, in some environments, fertilizers can have a negative effect on species diversity and in extreme cases may even pollute the surrounding environment (Fonseca et al., 1998; Hill & Heck, 2015; Zedler, 2000). ~~For example, nitrogen fertilization often contributes to coastal hypoxia and nitrous oxide emissions (Robertson & Vitousek, 2009). Therefore, consideration of the ecosystem, nutrient needs, and type of fertilizer is important to maximizing the benefits of fertilization approaches while minimizing the environmental impact of fertilizer use.~~ Therefore, consideration of the ecosystem, nutrient needs, and type of fertilizer is important to maximizing the benefits of fertilization approaches while minimizing the environmental impact of fertilizer use.

~~Balancing the positive and negative~~The ramifications of fertilizer use ~~is~~are especially relevant in coastal seagrass ~~ecosystem restoration. Seagrass ecosystems are~~systems, which are both important ~~coastal ecosystems~~habitats and currently facing global declines due to~~ direct~~ human disturbance and climate change (Bayraktarov et al., 2016). Seagrasses are a comparatively difficult and expensive coastal ecosystem to restore, partially due to eutrophication, competition from algae and other nutrient related issues (ibid). However, fertilizers have been consistently found to improve seagrass health and restoration success ~~(Armitage et al., 2011; Kenworthy et al., 2018)~~(Armitage et al., 2011; Kenworthy et al., 2018). ~~Therefore, it is critical to provide a fertilizer that directs nutrients toward seagrass growth and minimizes the release of nutrients to the surrounding environment.~~

Traditionally, both the direct application of controlled release fertilizers (Armitage & Fourqurean, 2016; Fonseca et al., 1998; Peralta et al., 2003; Sheridan et al., 1998) and the deployment of bird roosting stakes (Fonseca et al., 1994; Furman et al., 2019) have positive effects on seagrass ~~above and belowground~~ biomass~~ in multiple systems. Ecosystem~~, and can accelerate ecosystem succession for seagrass~~ also appears to be accelerated by the addition of nutrients in the short and long term~~ (Bourque & Fourqurean, 2014; Armitage et al., 2011). However, the use of traditional fertilization techniques in seagrass restoration may result in variable levels of nutrients or over-fertilization ~~(Fonseca et al., 1998; Kenworthy et al., 2018)~~(Fonseca et al., 1998; Kenworthy et al., 2018), with consequences for the succession of seagrass species (ibid).

One of the main issues with fertilization in aquatic seagrass systems is~~ the difficulty~~ that immersion and hydrodynamics can lead to rapid dissolution of fertilizers, increasing short term nutrient availability to the desired plant species, but at the expense of nutrient loss, ecosystem disruption, and pollution (Fonseca et al., 1998; Hill

& Heck, 2015; Olsen & Valiela, 2010). For example, Hall et al. (2006) had to replace buried fertilizer pellets every three to four months in a macrophyte restoration effort, while Herbert and Fourqurean (2008) found that bird ~~stake fertilization~~stakes (bird roosting structures that promote feces accumulation, Fonseca et al., 1994; Furman et al., 2019) can overfertilize seagrass ~~restoration~~ sites, disrupting succession and increasing epiphytic biomass. These drawbacks are due either to the fertilizers being adapted for terrestrial applications, releasing nutrients too rapidly after flushing with water, or in the case of bird stakes, due to variable rates of feces deposition combined with diffusion of nutrients in the water during precipitation and settling (Hill & Heck, 2015). Applying multiple doses of traditional mineral fertilizers ~~incurs a significant financial and labor cost~~ (Ferdie & Fourqurean, 2004; Hall et al., 2006; Olsen & Valiela, 2010)~~. Similarly, the~~ or monitoring bird stake ~~approach requires extra labor to monitor~~treated beds for symptoms of excess fertilization ~~and remove the stakes after about 18 months (Kenworthy et al., 2018). Thus, a slower dissolving fertilizer resistant to leaching may reduce overfertilization with less labor inputs while still providing benefits toward seagrass growth and survival.~~(Kenworthy et al., 2018) also incurs a significant financial and labor cost. Thus, a slower dissolving fertilizer that resists leaching may reduce overfertilization and labor expenses while still providing benefits toward seagrass growth and survival.

Struvite (magnesium ammonium phosphate, or $MgNH_4PO_4 \cdot 6H_2O$) is a by-product of wastewater treatment that is harvested in separated, side-stream sludge management processes (Ghosh et al., 2019). ~~Struvite forms when equal molar ratios of Mg^{2+}, NH_4^+, PO_4^{3-} occur in the solution, thus the feeding sources are typically nutrient-~~

~~rich sludge dewatering liquors or digestate often dosed with an external source of magnesium (Kumar & Pal, 2015; Martí et al., 2010).~~ Struvite is poorly soluble in water, but releases P more rapidly in the presence of organic acids exuded from roots, making it a potentially ideal fertilizer for direct plant uptake (Cabeza et al., 2011; Robles-Aguilar et al., 2019). Past studies have supported both high performance of struvite for terrestrial plant applications as well as its resistance to flushing (Lee et al., 2009; Rahman et al., 2014). ~~Struvite application for restoration purposes would also support a more sustainable wastewater management through the increased use of recovered resources (Mayer et al., 2016) and introduced restoration activities into a circular economy.~~

While the utilization of struvite in aquatic systems appears very promising, to date there is an absence of studies investigating this fertilizer in marine restoration projects, especially in combination with other fertilization techniques. While it has been demonstrated that struvite is poorly soluble fertilizer except when exposed to acidic conditions (Cabeza et al., 2011; Talboys et al., 2016), experiments determining the availability of struvite to submerged aquatic vegetation do not currently exist. Thus, the goals of this study were to 1) assess potential differences in seagrass performance (~~e.g. metrics like~~ shoot count, growth, length, and biomass as defined by Arrington, 2008, Herbeck et al., 2014, Rezek et al., 2019, Short & Coles, 2001, and Thomsen et al., 2012~~, among others)~~) after addition of struvite versus a polymer coated, ~~‘slow release’~~controlled release fertilizer (~~PCF,~~ Osmocote^{TM}) commonly used in seagrass restoration, and 2) to determine shifts in sediment and porewater nutrients caused by the introduction of the fertilizers in plots with and without seagrass. We hypothesized that seagrass in plots fertilized with struvite would have increased performance compared to plots fertilized with Osmocote^{TM}, and that struvite would be dissolved at a slower rate than Osmocote^{TM} (~~determined by measuring~~based on porewater total dissolved nutrients).

2. ~~2.~~ Materials and Methods

2.1. ~~2.1~~ Site Description and Design

To minimize the variability found in field experiments and more accurately investigate nutrient levels related to fertilization, a mesocosm experiment was conducted at the Whitney Laboratory of Marine Biosciences in St. Augustine, FL. Seawater (filtered through a shelly sand and activated charcoal biofilter) pumped from offshore entered a 6.5 m diameter mesocosm (approximately 1 m deep), to emulate the natural environment. Water flow was constant into the mesocosm. Experiments were based on the methods explained in the propagation guide for *Halodule wrightii*~~, prepared by the University of Southern Mississippi~~ (Biber et al., 2013). Seagrass was collected directly from donor sites off St. Martins Marsh Aquatic Preserve, FL. Shoots were removed from the donor sediment and maintained in cool conditions until they were transplanted into plastic pot containers (10 cm depth), buried in approximately 5 cm of coarse, shell-dominated sand taken from the local St. Augustine area (rinsed to reduce organics and residual nutrients). The sediment used ~~comprised at least 99% sand with~~had a mean grain size of 706 microns (not including particles greater than 2 mm).

2.1.1 Mesocosm Conditions

~~2.2.~~Mesocosm temperature and salinity remained between 27-31 °C and 33-38 parts per thousand respectively during the periods sampled (between 9 am and 3 pm)

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for both studies. The hydraulic residence time was variable at 0.5-2 days, due to a limited saltwater supply. The mean TDN of surface water was 0.44 ± 0.06 mg N L^{-1}, while the mean TDP was 0.035 ± 0.001 mg P L^{-1} (or 0.029 mg P L^{-1} when excluding a day of low inflow). The level of flow was great enough to prevent significant cross contamination of the plots studied, as well as prevent significant swings in temperature and salinity that could stress the plants.

2.2. Experiments

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Two separate experiments were conducted in the summer and fall of 2018. The first 60-day experiment consisted of six different treatment options, including bare sand with or without fertilizers (terrestrial ~~PCF~~polymer coated fertilizer or struvite) and seagrass with or without fertilizers. A second 70-day experiment was conducted consisting of multiple lower doses of both fertilizers.

2.2.1. ~~2.2.1~~ Single Dose/First Experiment

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For the ~~PCF~~polymer coated controlled release fertilizer treatment, Osmocote™ 14:14:14 NPK (Scotts Miracle-Gro Company, Marysville, OH, USA) was chosen due to its commercial availability, composition (containing both N and P), and past use in seagrass restoration experiments (Peralta et al., 2003; Sheridan et al., 1998; Tanner & Parham, 2010). Struvite used in the experiment was produced in a pilot scale fluidized bed reactor fed with sludge dewatering liquor. Detailed morphological and elemental characteristics are described elsewhere (Bydałek et al., 2018). Unlike the mostly homogenous struvite, each Osmocote™ prill has a porous outer layer that gradually releases a contained water-soluble nutrient dose through diffusion. The composition of elements is also different between the two compounds; with NH_4^+/NO_3^--N comprising

14% of Osmocote^{TM} versus NH_4^+-N comprising only 6% of struvite (Osmocote^{TM} manufacturer information, Kenworthy & Fonseca, 1992; Rahman et al., 2014). The P composition of both fertilizers is also different, with struvite (13% P as PO_4^{3-}) versus having a higher concentration by weight versus Osmocote^{TM} (~~13% P as PO_4^{3-} versus~~ 6.1% P as P_2O_5) (Osmocote^{TM} manufacturer information, Rahman et al., 2014).

In total, there were 30 plots, with an unplanted, untreated/unfertilized control (labelled control, n= 4), sediment-only treatments (labelled Control-Osmo and Control-Struv, n= 4), and seagrass control and treatments (labelled Seagrass, Seagrass-Osmo, and Seagrass-Struv, n= 6). Nutrient treatments were fertilized ~~with approximately~~by adding the Osmocote^{TM} or struvite equivalent of 3 g of P mixed into approximately 6 kg of sand (equivalent to 0.5 mg P g^{-1} DW sand), which was about half of what was considered “~~lightly~~low fertilized” according to Peralta et al. (2003~~). Each seagrass plot had exactly three individuals, each with five shoots. The first experiment was conducted for 60 days (Figure A.1). During this period, the levels of dissolved total P~~). The dosing was equilibrated to P as tropical seagrass systems are primarily P limited (Brodersen et al., 2017; Gras et al., 2003). In this experiment, serving as pilot study, N concentrations were not equilibrated, however given the actual fertilizer dosages, concentrations were still below the low fertilized treatment in Peralta et al.’s study (0.23 mg N g^{-1} DW sand for struvite and 1.16 mg N g^{-1} DW sand for Osmocote respectively). Each seagrass plot had exactly three individuals, each with five shoots. The first experiment was conducted for 60 days. During this period, the levels of dissolved total P porewater concentrations were excessively high, exceeding 100 mg P L^{-1} in the Osmocote^{TM} treatments and 5 mg P L^{-1} for struvite.

2.2.2. ~~2.2.2.~~ Multi-Dose/Second Experiment

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In this second experiment, struvite doses were 0.0125 (low dose struvite or Seagrass-Struv-Lo), 0.025 (medium dose struvite or Seagrass-Struv-Med) and 0.05 mg P g^{-1} DW sand (high dose struvite or Seagrass-Struv-Hi). For Osmocote^{TM}, 0.0125 (low dose Osmocote^{TM} or Seagrass-Osmo-Lo) and 0.025 mg P g^{-1} DW (medium dose Osmocote^{TM} or Seagrass-Osmo-Med) doses were used. Unplanted, fertilized controls had a 0.0250 mg P g^{-1} DW dose of Osmocote^{TM} (Osmocote^{TM} control or Control-Osmo) and struvite (struvite control or Control-Struv). Unfertilized, unplanted plots were labelled “control” while unfertilized, planted plots were labelled “unfertilized seagrass” or “Seagrass-Control”. There were four replicates for all controls/treatments. A high dose of Osmocote^{TM} was not used due to space limitations in the mesocosm and concerns of overfertilization based on the results of the single dose/first experiment. There were three individuals with five shoots per plot (initially two individuals with the third added 10 days post deployment to match the starting shoot count of the previous experiment).

2.3. ~~2.3~~ Plant and Nutrient Measurements

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Seagrass shoot count (seagrass shoots defined as a unit of several leaves or blades according to Short & Coles, (2001)), were quantified ~~in both experiments~~ approximately every 10 days in both experiments. During the second experiment, blade/leaf lengths (substrate to leaf tip according to Arrington, (2008)) were also quantified. Surface water was sampled for temperature, salinity, and total dissolved nutrients (Total Dissolved N/TDN, Total Dissolved P/TDP), while porewater was only sampled for total nutrients and (randomly) sulfide presence. Surface and porewater samples were collected using a syringe sampler fashioned out of a 60 mL syringe attached to a plastic tube and 1 mL serological ~~1 mL~~ pipette with an attached air stone.

The samples were filtered through a 0.45 μm~~ pore size~~ filter (Whatman, Maidstone, United Kingdom), preserved with sulfuric acid to a pH < 2, and stored at 4 ºC until analysis in the Wetland Biogeochemistry Laboratory (USEPA, 1974, 1993). Porewater was also tested for the presence of sulfide~~, which is toxic to seagrasses~~ (Calleja et al., 2007; Carlson et al., 1994) using a Hach ~~testing~~test kit (product number 2537800). No measurable sulfide was found in any plots sampled (detection limit 0.1 mg L^{-1}). DOC and TDN samples were analyzed on a Shimadzu TOC-L analyzer fitted with a N module (Shimadzu Scientific Instruments, Durham, NC, USA) according to EPA method 415.1 for TOC and ASTM D 8083 for total nitrogen (TN) (ASTM International, 2016; Nevins et al., 2020; USEPA, 1974). TDP was digested with persulfate in an autoclave and analyzed via a Shimadzu UV-1800 spectrophotometer (Shimazdu Corporation, Kyoto, Japan) using EPA method 365.1 ~~(Tootoonchi et al., 2018; USEPA, 1993)~~(Irick et al., 2015; USEPA, 1993).

At the end of the experiment, plant biomass and sediment were destructively sampled. Plants were rinsed to clean off sediments, and promptly frozen. ~~Once at~~In the lab, tissue samples were cleaned of epiphytes and rinsed with de-ionized water. Plant tissue and sediment samples were dried for 72 hours at 65 ºC~~,~~ and ground using a ball mill~~, and ~~. Sediment was analyzed for total carbon (TC), and nitrogen (TN), while tissue was analyzed for TC, TN, and phosphorus (TP). Bulk sediment TC/TN were run on an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA) ~~(dry combustion method)~~ (Nevins et al., 2020). Tissue TP was determined by ashing the sample followed by dissolution with 6 M HCL (following Andersen, 1976) and analysis for soluble P using a Shimadzu UV-1800 spectrophotometer (Shimazdu

Corporation, Kyoto, Japan) ~~(Liao et al., 2016; USEPA, 1993).~~(Liao et al., 2019; USEPA, 1993). Due to low and variable weights found after drying seagrass samples, plant dry biomass was calculated using a 10% wet weight conversion used for *H. wrightii* and *Thalassia testudinum* in Heck et al., (2015) and outlined in Short & Coles, (2001). A sediment particle analysis was also conducted to determine the distribution of particle sizes and possible changes over time. These samples were analyzed by the Soil and Water Sciences Environmental Pedology and Land Use Laboratory using laser diffraction (LD) with a Beckman Coulter LS-13320 multi-wave particle size analyzer (Beckman Coulter Diagnostics, Brea, CA, USA).

2.4. ~~2.4~~ Statistical Analyses

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Differences in seagrass metrics (shoot count and shoot length) and porewater nutrients for both experiments were calculated using a linear mixed model, followed by a post hoc multiple comparison significant (Fisher's Least Significant Difference test). Factors included the treatment type, date, and the interaction between treatment and date. A linear mixed model analysis was also conducted on sediment and biomass measurements from the second experiment, testing the effect of treatment type. The tests were run using JMP 15.2.1 (SAS Software, Cary, NC, USA) with significance set to α= 0.05. To determine the fit of the model predictions to the measured data, residuals and qq-plots were visually inspected and data was log transformed as necessary (shoot counts, shoot lengths, and total dissolved nutrients). To differentiate between the effects of fertilization methods, K-means clustering was applied to classify all observations in the multi-dose/second experiment. K-means were computed using the `kmeans` function in R (version R-4.0.2.). Given the number of observations (n= 6) the data was predefined into two clusters (`centers= 2`). Prior to the analysis, the data was standardized using the `scale` function (each element is subtracted by the mean value of the vector and divided by standard deviation of the vector). The results were visualized using the `fviz_cluster` function (factoextra package) based on function's encoded principal component analysis (PCA) ~~(Kassambara & Mundt, 2017)~~(Kassambara & Mundt, 2017).

3. ~~3.~~ Results

~~2.1.1 3.1 Mesocosm Conditions~~

~~Mesocosm temperature and salinity remained between 27-31 °C and 33-38 ppt respectively during the periods sampled (between 9 am and 3 pm) for both studies. The hydraulic residence time was variable at 0.5-2 days, due to a limited saltwater supply. The mean TDN of surface water was 0.44 ± 0.06 mg N L^{-1}, while the mean TDP was 0.035 ± 0.001 mg P L^{-1} (or 0.029 mg P L^{-1} when excluding a day of low inflow). The level of flow was great enough to prevent significant cross contamination of the plots studied, as well as prevent significant swings in temperature and salinity that could stress the plants.~~

3.1. ~~3.2~~ Single Dose/First Experiment

3.1.1 Plant Metrics

Increases in shoot counts occurred one month after transplantation for the struvite treatment. However, this was not the case with the unfertilized control or the Seagrass-Osmo treatment, which both slowly declined on average. At the end of the first experiment, mean ~~seagrass~~ shoot counts ranged from 6.33 ± 0.87 shoots in the Seagrass-Osmo treatment to 52.33 ± 5.49 shoots in the Seagrass-Struv treatment (Figure 1). ~~The effects of fertilizer treatment, date, and the treatment x date interaction were significant for the shoot count (Table 1).~~ Seagrasses in ~~Seagrass-Struv~~struvite

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fertilized plots had significantly higher shoot counts than the seagrass control and ~~Seagrass-Osmo overall (t ≥ 6.83,~~ Osmocote treatment (p < 0.~~0001), while there were no significant differences between the seagrass control and Seagrass-Osmo plots.~~01). More specifically, the Seagrass-Struv treatment had a significantly higher shoot count in mid-July, just one month after planting (~~t= 2.30,~~ p~~=~~ < 0.~~0243~~05), becoming greater over the next month (by end of the study ~~t= 19.71,~~ p < 0.~~0001~~001). By the end of the study, the unfertilized seagrass also had a significantly higher number of shoots than the Seagrass-Osmo treatment (t= 2.56, p~~=~~ < 0.~~0124~~05). ~~The effects of treatment, date, and the interaction between treatment and date were significant for porewater TDP (Table 1).~~

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3.1.2 Water Chemistry

The TDP levels were significantly higher in the Seagrass-Osmo plots than the unfertilized controls and Seagrass-Struv treatments (~~t > 15.12,~~ p < 0.0001, table S1). By the end of the study, the average TDP concentration for the Seagrass-Osmo porewater plots was 136.09 ± 15.71 mg P L^{-1} for the unplanted plots (Control-Osmo) and 109.53 ± 19.96 mg P L^{-1} for the planted plots (Seagrass-Osmo), more over ten times higher than the struvite plots, which was 2.43 ± 0.61 mg P L^{-1} in the unplanted plots and 0.76 ± 0.19 mg P L^{-1} in the Seagrass-Struv plots.

Porewater TDP in the Control-Struv treatment was significantly higher than the control, unfertilized seagrass, and the Seagrass-Struv treatments (~~t > 4.72,~~ p < 0.~~0001~~001), indicating that significant uptake of TDP by seagrasses likely occurred. There were no significant differences in TDP between the unplanted and planted Seagrass-Osmo plots, overall or during any specific sampling date. ~~Over time, TDP concentrations in both the Control-Struv and Seagrass-Struv treatments significantly~~

~~increased by the end of the study ($t > 2.44$, $p < 0.02$) (Figure 1). Overall, the concentration of TDP in the Seagrass-Osmo plot porewater significantly increased over time ($t > 4.29$, $p < 0.0001$), with the Seagrass-Osmo fertilized plots increasing significantly in TDP between the first and second sample dates ($t > 3.74$, $p \leq 0.0005$). Subsequent sampling periods showed no statistically significant changes in TDP concentrations for the Control-Osmo or Seagrass-Osmo plots over time. There were no significant differences within or between the control and unfertilized seagrass plots.~~

3.2. ~~3.3~~ Multi-Dose/Second Experiment

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3.2.1. Plant Metrics

At the end of the second experiment, the average seagrass shoot counts ranged from 8.00 ± 0.41 shoots in the Seagrass-Control to 14.50 ± 3.10 shoots in the ~~Segrass~~Seagrass-Struv-Med treatment (Figure 2). There was relatively less growth in the second experiment versus the first/single dose experiment, however the effects of date and its interaction with the treatment type were still significant for shoot count (Table ~~2). The shoot count of the Seagrass-Control was significantly lower than the Seagrass-Osmo-Lo treatment ($t= 2.61$, $p= 0.0117$), Seagrass-Osmo-Med ($t= 3.01$, $p= 0.0040$), and the Lo/Med/Hi doses of struvite ($t= 3.88$, $p= 0.0003$, $t= 3.88$, $p= 0.0003$, and $t= 3.06$, $p= 0.0034$ for the Lo, Med, and Hi doses, respectively) during the 10/05 or Day 31 sampling. During the final sampling period (10/25 or 74 days after deployment), struvite plots had significantly higher shoot counts than the Seagrass-Control ($t= 3.42$, $p= 0.0012$, $t= 3.50$, $p= 0.0009$, and $t= 3.35$, $p= 0.0015$, for the Lo, Med, and Hi doses, respectively), while there were not significant differences between Osmocote™ and the Seagrass-Control (Figure 2). In addition, there were no significant differences between~~

~~the struvite and Osmocote™ treatments for shoot count~~S-2). After 53 days, seagrass shoot count started showing signs of treatment effect in comparison to control seagrass plot which showed significant shoot count declines (p < 0.05) in comparison to the rest of the fertilized seagrass plots. By the end of the experiment (74 days) only the Seagrass-Struv-Med treated seagrass plots maintained plant density (14.50 ± 3.10 shoots) close to the original coverage of 15 shoots per plot indicating high transplantation survival rate. At the conclusion of the study, only the struvite fertilized plots were statistically higher in shoot count than unfertilized plots.

The effects of both treatment and date were significant for blade length (Table S-2). All fertilized treatments became significantly greater in length than the Seagrass-Control~~ during and~~ after 39 days post deployment (Figure 2). The average seagrass blade length ranged from 9.1 ± 1.02 cm in the unfertilized seagrass to 19.1 ± 1.74 cm in the medium dose struvite~~. Seagrass blade length in the Seagrass-Struv-Med was significantly higher than the Seagrass-Osmo-Lo (*t*= 2.84, *p*= 0.0065, *t*= 3.62, *p*= 0.0007, and *t*= 4.40, *p* < 0.0001 for the Seagrass-Osmo-Lo 39, 53, and 74 days after deployment, respectively) and Seagrass-Osmo-Med (*t*= 3.13, *p*= 0.0029, *t*= 3.07, *p*= 0.0036, and *t*= 3.02, *p*= 0.004 for the Seagrass-Osmo-Med at 39, 53, and 74 days after deployment, respectively). The Seagrass-Struv-Med treatment also had a significantly higher shoot length than the Seagrass-Struv-Lo 53 (*p*= 0.0276) and 74 days after deployment (*p*= 0.0257).~~ by the end of the experiment. The highest increase in blade length was observed in struvite treatments. The Seagrass-Struv-Med treatment showed a significantly (p < 0.005) higher blade growth than the Seagrass-Osmo-Lo/Med treatments.

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The mean aboveground biomass ranged from 0.012 ± 0.004 g DW in the Seagrass-Control to 0.080 ± 0.011 g DW in the Seagrass-Struv-Hi treatment (Figure ~~4~~3), with the effect of treatment type being significant (Table ~~3). For~~ S-3). All fertilized plots had significantly higher aboveground biomass~~, the Seagrass-Control was significantly lower~~ than the ~~Seagrass-Osmo-Med (*t*= 1.53, *p*= 0.045), low (*t*= 2.60, *p*= 0.018), medium (*t*= 4.07, *p*= 0.0007), and high dose struvite (*t*= 4.03, *p*= 0.0008) (Figure 3). Med and Hi dose struvite treatments were significantly higher in aboveground biomass than~~ control, except for the Seagrass-Osmo-Lo ~~(*t*= 2.54, *p*= 0.0205 and *t*= 2.50, *p*= 0.0225 for medium and high dose struvite). Marginal~~treatment ($p < 0.05$). There was a marginal significance ~~was also~~ found for the Med and Hi struvite ~~treatments~~doses having ~~a~~ higher aboveground biomass than the Seagrass-Osmo-Med (~~*t*= 1.92, *p*= 0.0713 and *t*= 1.87, *p*= 0.0775 for Med and Hi dose struvite, respectively).~~

$p < 0.08$). Belowground biomass ranged from 0.11 ± 0.02 g DW in the Seagrass-Control to 0.20 ± 0.01 g DW in the ~~high dose struvite~~Seagrass-Struv-Med treatment (Figure ~~4~~3). The ~~effect of treatment type was also significant for~~ belowground biomass of control plots was significantly lower compared to all fertilized plots except for the Seagrass-Osmo-Med (Table S-3). ~~The Seagrass-Osmo-Lo, Seagrass-Struv-Lo, and~~Additionally, the Seagrass-Struv-Med ~~were~~dose had significantly higher ~~than the Seagrass-Control (*t*= 2.47, *p*= 0.0238, *t*= 2.57, *p*= 0.0194, and *t*= 3.98, *p*= 0.0009, for~~ belowground biomass compared to the Seagrass-Osmo-~~Lo,~~Med and Seagrass-Struv-~~Lo, and Seagrass-Struv-Med, respectively). Additionally, the Seagrass-Struv-Med was significantly higher than both the Seagrass-Struv-Hi (*t*= 2.24, *p*= 0.0378) and the Seagrass-Osmo-Med (*t*= 2.93, *p*= 0.0089).~~

~~At the end of the experiment, the mean porewater TDN concentration ranged from 1.68 ± 0.15 mg N L^{-1} in the Seagrass-Control to 17.26 ± 4.98 mg N L^{-1} in the Seagrass-Osmo-Med (Table A.1). Only the effect of the treatment type was significant for TDN (Table 2). Overall, the porewater TDN concentrations for the Control-Osmo and planted Seagrass-Osmo-Med were significantly higher than the controls and the struvite treatments ($t \geq 2,06$, $p \leq 0.0423$). The Seagrass-Osmo-Lo was significantly higher in TDN than all treatments except the Seagrass-Osmo-Med and the Seagrass-Struv-Hi treatment ($t \geq 2.01$, $p \leq 0.0473$). All struvite doses were significantly higher in TDN than both the unplanted control and Seagrass-Control ($t \geq 3.62$, $p \leq 0.0005$).~~

~~The mean porewater TDP concentrations ranged from 0.084 ± 0.021 mg P L^{-1} in the Seagrass-Control to 0.551 ± 0.105 mg P L^{-1} in the Seagrass-Osmo-Med at the end of the experiment. The effects of treatment and date were significant for porewater TDP (Table 2). Similarly, the unplanted control and Seagrass-Control had significantly lower TDP than all fertilized plots ($t \geq 4.68$, $p \leq 0.0001$). The Seagrass-Osmo-Med was significantly higher than all other controls/treatments except the equivalently dosed unplanted Osmocote^{TM} treatment ($t \geq 3.38$, $p \leq 0.001$), while Seagrass-Osmo-Lo was significantly higher than the controls and the equivalently dosed (P basis) struvite treatment ($t \geq 4.05$, $p \leq 0.0001$). Marginal significance was also found for the Seagrass-Osmo-Lo being higher in porewater TDP than the Seagrass-Struv-Med ($t = 1.92$, $p = 0.0569$). Over time, TDP concentrations appeared to fluctuate greatly between fertilized treatments, while remaining stable for unfertilized controls. Between the two highest peaks (Day 6 and Day 31), Osmocote^{TM} plots had a significant reduction in porewater~~

~~TDP concentration (t= 3.06, p= 0.0028, t= 2.81, p= 0.0059, and t= 2.44, p= 0.0164 for the Osmo-Control and Lo/Med Osmocote^{TM} treatments, respectively).~~

Hi doses ($p < 0.05$). Aboveground tissue %TN ranged from 1.9% in the unfertilized seagrass (one sample) to 2.34 ± 0.23% in the Seagrass-Struv-Lo treatment, while tissue %TP ranged from 0.236 ± 0.007% in the Seagrass-Struv-Lo treatment to 0.258 ± 0.016% in the Seagrass-Osmo-Lo treatment (Table ~~A.3~~S-7). There was no significant effect of treatment on aboveground %TN or %TP (Table 4). ~~This lack of significant difference is possibly due to the absence of available control replicates. For example, the aboveground control only had a single combined sample (from n=4).~~ The mean aboveground N:P ratios ranged between 8.3 ± 0.57 for the Seagrass-Osmo-Lo and 10.0 ± 1.20 for Seagrass-Struv-Lo treatment. The N:P ratio and the mean aboveground TN and TP weights in the seagrasses (calculated by multiplying the biomass with the tissue %TN or %TP) yielded no significant differences (Tables ~~5~~S-4 and ~~A.3).~~

7). Belowground tissue %TN ranged from 0.53 ± 0.07% for the Seagrass-Osmo-Lo treatment to 0.84 ± 0.06% in the Seagrass-Osmo-Med treatment, while tissue %TP ranged from 0.154 ± 0.009% for the Seagrass-Osmo-Lo treatment to 0.179 ± 0.008% for the Seagrass-Osmo-Med treatment~~ (Table A.3).~~. The effect of treatment type was significant for belowground %TN (Table 4), with the Seagrass-Osmo-Med being significantly higher than the Seagrass-Osmo-Lo (~~t= 3.43, p=~~< 0.~~0037~~05). No effects were significant for belowground %TP. The mean belowground N:P ratio ranged from 3.4 ± 0.47 for the Seagrass-Osmo-Lo and 4.7 ± 0.36 for the Seagrass-Osmo-Med treatment~~ (Table A.3). Similarly, no significant differences in the belowground N:P~~

~~ratios were found. However, the~~. The effect of treatment type was significant for both the belowground mass of TN and TP (% total nutrient x biomass, Table ~~5). For belowground TN weight, the Seagrass-Struv-Med was significantly higher than the Seagrass-Control (t= 3.38, p= 0.0041) and the Seagrass-Osmo-Lo (t= 2.66, p= 0.0177). For the belowground TP weight, the Seagrass-Struv-Med was significantly higher than the Seagrass-Control (t= 3.67, p= 0.0023) and the Seagrass-Osmo-Med (t= 2.22, p= 0.0421). Additionally, the Seagrass-Struv-Lo was significantly higher than the Seagrass-Control (t= 2.59, p= 0.0205).~~S-5).

3.2.2. Water, Tissue, and Sediment Chemistry

Nutrient dynamics in porewater differ significantly between the fertilizer types indicating different dissolution kinetics and plant and substrate interaction. Unfertilized control plots (planted and unplanted) showed variable TDN concentrations throughout the experiment however, never surpassing 2 mg TDN L^{-1}. Background porewater TDP content in observed controls varied within 0.05-0.15 mg TDP L^{-1}. The biggest nutrient release was observed at plots fertilized with Osmocote with peak nutrient concentrations occurring at 6th day of experiment reaching 26.8 ± 7.53 mg TDN L^{-1} and 17.68 ± 6.74 mg TDP L^{-1} for medium Osmocote dose. TDP dynamics in struvite seagrass treatments were highly variable throughout the time and showed alternating pulses of TDP release. However, by the end of the experiment porewater TDP content in struvite fertilized plots was 2-3 times lower than in respective Osmocote treatments. DOC measured at the end of the study was between 12.26 ± 0.67 mg DOC L^{-1} for Seagrass-Struv-Lo and 14.71 ± 1.23 mg DOC L^{-1} for Seagrass-Osmo-Lo.

The average TC content of sediment ranged from 48.7 ± 5.02 g C kg^{-1} in the medium dose struvite to 58.2 ± 5.63 g C kg^{-1} in the Seagrass-Struv-Hi, while the average TN content ranged from 2.02 ± 0.032 g N kg^{-1} in the Seagrass-Control to 2.10 ± 0.020 g kg^{-1} in the Seagrass-Struv-Hi treatment (Table ~~A.5). The TP content of sediment was not measured due to the high variability of replicates (possibly caused by the large grain size of the sediment and/or the granular nature of the fertilizers, creating regions of low/high nutrients).~~S-6). There were no significant differences in the TC or TN contents between treatments (Table ~~A.6~~S-7).

~~Nutrient dynamics (TN, TOC and TDP), above and belowground biomass, and shoot count data~~Porewater nutrients and seagrass metrics were used to further assess the global effect of fertilization dose and method based on multivariate analysis. K-means clustering detected two separate groups. The struvite treatment was clearly distinguished from the Osmocote^{TM} treatment and control plot, occupying separated, non-overlapping clusters on the PCA plane (Figure 4), reinforcing the significant effects of struvite on seagrass and its surrounding environment.

4. ~~4.~~ Discussion

4.1. ~~4.1~~ Factors in Seagrass Performance

~~In all but the Seagrass-Osmo in the first experiment, fertilizer~~Fertilizer application improved seagrass metrics compared to the unfertilized control~~, including~~ in all but the Seagrass-Osmo treatment of the first experiment. This included average shoot count (more than six times higher vs the control at the end of the first experiment, and ~~41% or more~~up to 81% at the end of the second experiment), length (~~32% or greater~~up to 110% at the end of the second experiment, Figure 2), and biomass (~~52% or greater~~up to 138% at the end of the second experiment, Figure 3). In general, these results support

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past findings examining the effects of fertilizer in the restoration ~~techniques in~~of seagrass ecosystems ~~(Armitage et al., 2011; Kenworthy et al., 2018).~~(Armitage et al., 2011; Kenworthy et al., 2018). Additionally, the results of this study found that compared to equivalent P dosages with Osmocote, fertilization using struvite resulted in higher average seagrass shoot count (more than ~~six~~eight times higher by the end of the first experiment, and ~~60% or more compared to the Seagrass-Struv-Med~~29% at the end of the second experiment), length (~~36% or more compared to the Seagrass-Struv-Med~~up to 36% at the end of the second experiment, Figure 2), and biomass (~~10-~~up to 60% higher total biomass ~~compared to the Seagrass-Struv-Med~~ at the end of the second experiment, Figure 3~~) compared to equivalent doses of Osmocote™.~~). The significant multivariate improvements in plant metrics ~~produced~~ in both experiments are promising towards the use of struvite as a fertilizer to rapidly establish seagrass species in future restoration efforts.

In addition to improving seagrass metrics, struvite consistently ~~appeared to release~~released less nutrients than Osmocote™. Porewater TDN was excessive in the Osmocote™ treatment in the first experiment ~~(the sample readings were out of range, x > 10~~(> 100 mg/L~~ without dilutions).~~). In the second experiment, TDN in struvite ~~introduced TDN~~ treated plots was ~~only~~as low as 12% of Osmocote™ ~~released TDN experiment~~treated plots (Table S-~~1~~8). Porewater TDP in equivalent struvite doses was less than 2% TDP of Osmocote™ in the first experiment (Figure 1), and as low as 10% P of Osmocote™ in equivalent struvite doses in the second experiment (Table S-~~1~~8). The speed of nutrient release by Osmocote™ was so high, that it may have contributed to the decreased performance of the Osmocote™ treatments through excessive N levels, as evidenced by roots that appeared stunted from possible root burn (observed in the first experiment, Figure S-1), commonly associated with N exposure (NC State, 2018; Schönau & Herbert, 1983). ~~This~~

The possible root burn in Osmocote$^{\text{TM}}$ treated seagrass may be the result of nitrate ~~specifically, as it is included in the Osmocote$^{\text{TM}}$ blend and was found to inhibit seagrass biomass in past fertilization studies~~ (Peralta et al., 2003; Statton et al., 2014)~~.~~ ~~Alternatively, toxicity may have been caused by the ammonia fraction in the Osmocote, as NH$_x$ forms are also toxic to seagrasses, especially at low biomass levels~~ or ammonia (van der Heide et al., 2008)~~.~~ fractions in the fertilizer. However, previous seagrass (*Zostera marina*) mesocosm studies have detected increased seagrass metrics following Osmocote$^{\text{TM}}$ fertilization. For example, *Zostera marina* plants were found to have increased shoot counts after one month of Osmocote$^{\text{TM}}$ 14:14:14 NPK fertilizer exposure compared to unfertilized plots (Wang et al., 2020). Similarly, another study found significant differences in shoot length in *Z. marina* over a period of two months when exposed to fertilizer doses higher than those used in this study (Peralta et al., 2003). In these cases, it should be noted that *Z. marina* exhibited a “remarkable tolerance” of N and P fertilization, and many species of seagrass may not be as flexible regarding higher levels of nutrient exposure.

Another factor affecting the difference between struvite and Osmocote$^{\text{TM}}$ could be the balance of N versus P. In the second experiment, the aboveground tissue N:P ratios (8.3 ± 0.57 to 10.0 ± 1.20, Table S-9) consistently exceeded the traditionally accepted threshold for a balanced nutrient supply~~. The mean N:P ratios ranged between 8.3 ± 0.57 to 10.0 ± 1.20 (Table S-2), while a~~ for seagrasses (14 weight N:P ratio calculated from the 30:1 molar N:P ratio as provided by Atkinson & Smith, [1983~~] is considered balanced for seagrass.~~]). A study of *H. wrightii* found that in a natural system (Florida Bay) the molar N:P was over 20, while in ~~an enriched scenario (~~a fertilized ~~with~~scenario (using bird roosting stakes) the ratio was approximately 13 (Powell et al., 1989). Thus, the authors argued that *H. wrightii* was P limited in ~~an unenriched~~a natural setting, and N limited ~~in the enriched setting.~~when fertilized. Another study in Florida Bay found that *H. wrightii* was “released” from P limitation ~~had~~at tissue ~~weight to~~N:P weight ratios~~ of~~ between 9.7 and 21 (Armitage et al., 2011). Generally, the *H. wrightii* in all fertilized plots did not appear to be strongly limited by a specific nutrient, exceeding the 1.8% TN/ 0.2% TP tissue nutrient requirement defined by Duarte (1990). The exception to this may have been the control, which was closer to N limitation than all plots with a 1.9% TN tissue content, although this conclusion is tenuous because only one replicate was able to be analyzed due to a lack of biomass.

The lack of significant differences in tissue nutrient content between fertilized and non-fertilized treatments may be due to delays in nutrient response by the plants. For example, one study found that it took *Thalassia testudinum* four months to acquire elevated N levels after fertilizer exposure, while elevated P levels in plants took up to 14 months to develop (Ferdie & Fourqurean, 2004). While *H. wrightii* is a faster growing species, and higher growth was demonstrated in fertilized vs non-fertilized plots, the limited experiment duration may not have fully captured long-term increases in tissue content. However, significant differences in belowground nutrient content (i.e. medium dose struvite vs. non-fertilized control, Table S-4) and tissue nutrient weight ~~may~~(Table S-5) indicate uptake of nutrients by the seagrass.

~~The surface and porewater results appear to support N limitation of the mesocosm environment, with all controls/treatments having a TDN/TDP ratio of less than 20 (most notably porewater TDN/TDP ratio of the unplanted control at 12.7 ± 1.71, and the unfertilized seagrass at 10.0 ± 1.89, Table S-1). Thus, the results of the experiment and past studies appear to support the argument that seagrasses in this study were either nutrient balanced or slightly N limited. In this case, the likely lack of severe N limitation, combined with the high levels of porewater nutrients, potentially indicates that the N content of struvite (6% by weight) is sufficient to improve seagrass growth in this system.~~

Furthermore, the size of the mesocosm plots may have been a factor in the high porewater nutrient levels~~ found in the experiment~~ by preventing lateral flow of porewater and limiting diffusion. The current flow and increased sediment depth may dilute porewater, increase diffusion, and reduce the effectiveness of fertilizers in a natural environment, requiring more fertilizer for field studies. This potential problem may be partially compensated by the relatively large grain size of the shelly sand used in the study, compared to the often silty sand found in seagrass systems (a property produced by seagrass beds as discussed in Folmer et al., 2012). The lack of sulfide present in the experiment also indicates a higher redox potential that is likely not present in field experiments.

This study demonstrated that struvite and Osmocote™ both released N and P ~~unabated ~~for at least two months (Table S-~~1~~8). Based ~~off~~on longer studies~~ using Osmocote™~~, it is expected that ~~the ~~Osmocote™ would provide N and P for ~~a couple more months, totaling ~~4-6 months ~~based on ~~(Hall et al., ~~(~~2006~~) and~~; Olsen and Valiela~~ (~~, 2010). Struvite may be able to provide nutrients for longer periods, indicated by its slower release rate. After the second experiment, ~~the~~selected fertilized plots were moved to another mesocosm and left submerged. A year after the experiment was deployed, the only evidence found of the Osmocote^{TM} fertilizer were the outer membranes of the prills, whereas struvite granules were still found in the mesocosm plots, indicating a potential continued release of nutrients. ~~While~~Thus, while the effects of struvite were only measured for up to nine weeks, the presence of struvite after this extended period indicates that struvite could be effective throughout a whole growing season or longer. The ability of struvite to produce higher seagrass metrics while emitting less nutrients (indicating a more sustained release of nutrients over a longer period of time) is promising toward the future applications of struvite in future coastal restoration efforts.

4.2. Field Applications of the Study

The controlled environment of the mesocosm study allowed tests to be done with minimal interference from the confounding variables of a field study. However, several external factors may still have affected the results of the two experiments. The first experiment was conducted at the peak of the seagrass growing season (June through August), whereas the second experiment occurred during the end of the season (August through October, with the season typically ending in September; Choice et al., 2014). The later date of deployment could help explain why ~~the~~ differences between shoot counts were not as apparent in the second experiment~~ compared to the first.~~. Based on the declining seagrass performance ~~found when exceeding~~above the medium/0.025 mg P g^{-1} DW dose, there may have been even larger differences in the first experiment between struvite and Osmocote$^{\text{TM}}$ if the second experiment was begun earlier in the summer.

~~4.2~~When considering the broad applicability of the results, it is important to note how close the conditions in the mesocosm were mimicking the natural environment. First, the local sediment substrate was not sterilized and contained a representative microbial population. Similarly, seawater for the mesocosm was only prefiltered to minimize inputs of algae or debris, and largely maintained the natural composition and physiochemistry. The mesocosm environment was sheltered from hydrodynamic disturbance and herbivory which are significant problems in field restoration efforts (Bourque & Fourqurean, 2013; W. Kenworthy et al., 2018; Tuya et al., 2017). However, there are numerous techniques such as protective cages, or biodegradable lattices, artificial seagrass, in ground fertilizer application, and sediment tubes that aim to minimize environmental disturbances and which can be successfully integrated into restoration projects utilizing fertilizers (Hall et al., 2006; Hammerstrom et al., 1998; W. J. Kenworthy et al., 2018; Li et al., 2019; MacDonnell et al., 2022; Temmink et al., 2020; Tuya et al., 2017).

Multiple field and mesocosm seagrass studies investigating the use of Osmocote$^{\text{TM}}$ have yielded generally similar results (Peralta et al., 2003; Pereda-Briones et al., 2018; Tanner & Parham, 2010). Both struvite/Osmocote$^{\text{TM}}$ experiments could be considered extensions of these previous investigations with real world applications. However, it must be noted that a successful mesocosm scale study such as this one cannot simply be scaled up to field applications. Rather, it would require the additional understanding of local environmental conditions and applied restoration techniques that enhance the success rate. Therefore, a future field study would be recommended to optimize the dose of struvite in different biogeochemical conditions and assess associated operational efforts and costs.

4.3. Implications/Applications of Struvite

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The integration of struvite in restoration projects could have multiple advantages ~~concerning~~for both~~ future~~ environmental management and sustainability of wastewater treatment. First, more research is needed, but struvite is potentially less harmful for the environment than ~~traditionally available,~~traditional commercial fertilizers. For example, struvite is sourced from wastewater, a source of eutrophication for many coastal systems (Mayer et al., 2016). The N content of struvite is also relatively low, and while it still provides plants with nutrients, it limits excess fertilization and resulting nitrous oxide emissions (Rahman et al., 2014). Second, that struvite ~~has the potential to be a~~is sustainable~~,~~ and locally sourced ~~fertilizer. This could have~~has global implications as P resources are being depleted in an accelerating rate, and there are indications that demand will surpass supply within the next 20 years ~~(Nedelciu et al., 2020)~~(Nedelciu et al., 2020). The processing of struvite allows for the production of a P fertilizer without dealing with the instability and increasing costs of importing fertilizer ~~(Rufí-Salís et al., 2020; Ye et al., 2020)~~(Rufí-Salís et al., 2020; Ye et al., 2020). Finally, the feasibility of using struvite on multiple scales has been demonstrated in experiments and industrial applications, indicating a practical and readily available treatment process ~~(Ghosh et al., 2019).~~(Ghosh et al., 2019).

The advantages of struvite in reducing pollution and phosphate shortages, combined with its feasibility, make it an attractive ~~option as an~~ alternative P and N fertilizer. Struvite is a ~~widely~~ recognized slow~~-~~-release terrestrial nutrient amendment

~~characterized~~ with a low environmental footprint. However, struvite application~~ in agriculture~~ is still limited due to its high price in comparison to conventional mineral fertilizers and availability. Therefore, extending application of struvite into ~~non-agricultural applications~~areas such as restoration could potentially create a new market ~~and consequently lower the price~~, thus making struvite more affordable and available. This is particularly important since struvite represents a very important aspect of circular economy in water management.

5. ~~5.~~ Summary and Conclusions

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Because of the current need for effective fertilization methods that minimize environmental risk, this study evaluated the wastewater by-product struvite and its potential to enhance seagrass growth under simulated natural conditions. ~~Within the fertilizer types, seagrass~~Seagrass growth metrics (shoots, length, biomass) in plots fertilized with struvite were consistently equal to or better than ~~a common~~the commercial fertilizer Osmocote$^{\text{TM}}$. This improvement in seagrass performance was provided while also producing lower porewater nutrient release from equal P fertilization doses, likely due to the slower release of nutrients from struvite delivering a low but sustained load of N and P to the rhizosphere. Excessive N inputs from the Osmocote$^{\text{TM}}$ treatment in the first experiment may have even reduced performance of treated plots compared to the unfertilized control. Measurements of porewater nutrients and visual observations indicated that struvite has a lower solubility and is therefore longer lasting compared to Osmocote$^{\text{TM}}$ in marine conditions. Other possible factors in plant performance, including the effects of specific nutrients (i.e. temporal delays in N/P tissue concentration, micronutrient differences), current flow (possibly increasing nutrient diffusion), and sediment particle size (affecting dissolution rates and redox potential), will require further investigation.

~~In the future,~~Future studies should apply the results of this experiment~~ in the field~~ in multiple coastal systems, ensuring that results are not constrained to a seagrass mesocosm setting. Testing the solubility of struvite in different environments may reveal more applications for the fertilizer~~ in different environments. Ideally~~. Experiments should include other seagrass species with diverse nutrient requirements, and ideally, a restoration experiment would take place over multiple growing seasons to determine how long struvite remains effective. Special consideration should also be given toward testing the effectiveness of struvite ~~versus Osmocote™~~ in a ~~heavily~~more N~~-~~-limited environment, ~~as Osmocote™~~where other fertilizers may have ~~an~~a better advantage~~ due to higher N content~~. This study was a first ever attempt to apply struvite in marine restoration project, serving as an example of interdisciplinary ~~merge~~merger between wastewater treatment engineering and restoration ecology. The~~ obtained~~ positive results here should encourage future research and field activities to further explore ~~struvite~~the application of struvite and similar materials for restoration projects~~ in both terrestrial and aquatic environment~~.

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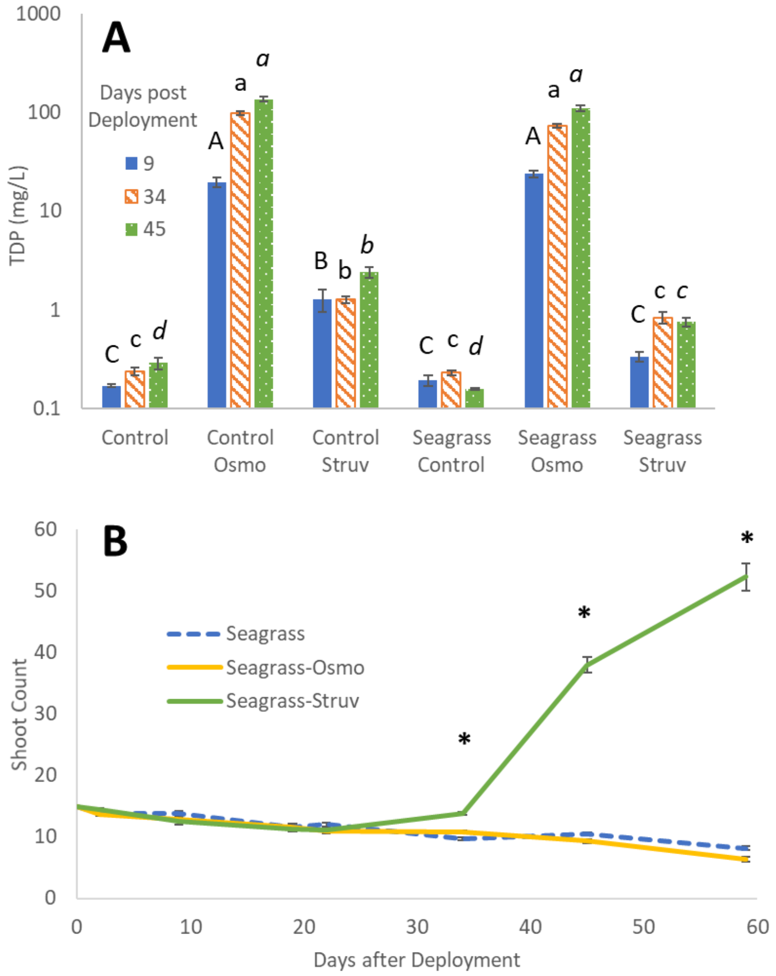

Figure 1. Porewater total dissolved phosphorus (TDP) (A), and seagrass shoot count (B) taken during the first mesocosm experiment. The treatments were labelled Seagrass (for the unfertilized seagrass plots), Seagrass-Osmocote™ (for planted plots fertilized with Osmocote™), and Seagrass-Struv (planted plots fertilized with struvite).

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The asterisks designate significant differences between treatments for the same sample dates. Points represent the mean of six replicates (± SE).

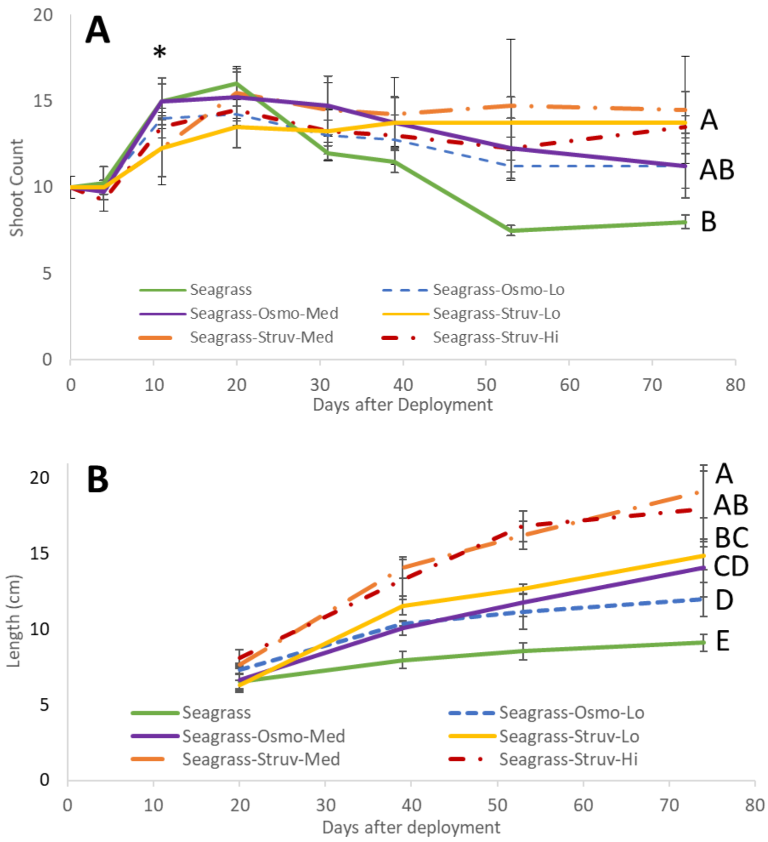

Figure 2. Shoot count (A) and blade length (B) from the second mesocosm experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). *: Five shoots were added to each plot to match the first/single dose

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experiment. Letters designate significant differences between treatments for the same sample dates. Points represent the mean of four replicates (± SE), except for above-ground biomass, which had two to four replicates.

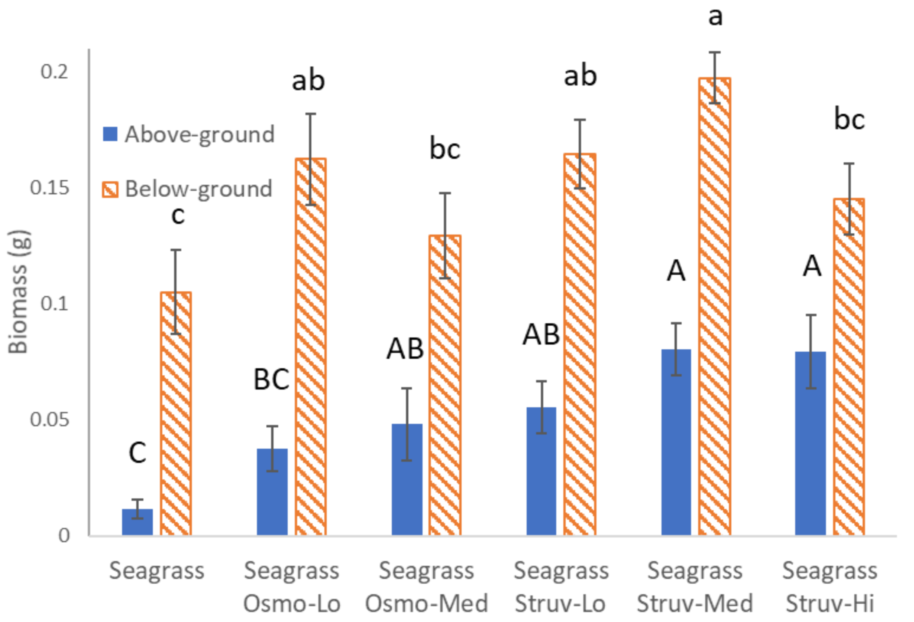

Figure 3. Above and belowground biomass from the second mesocosm experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote^{TM}), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Letters designate significant differences between treatments for the same sample dates. Points represent the mean of four replicates (± SE), except for above-ground biomass, which had two to four replicates.

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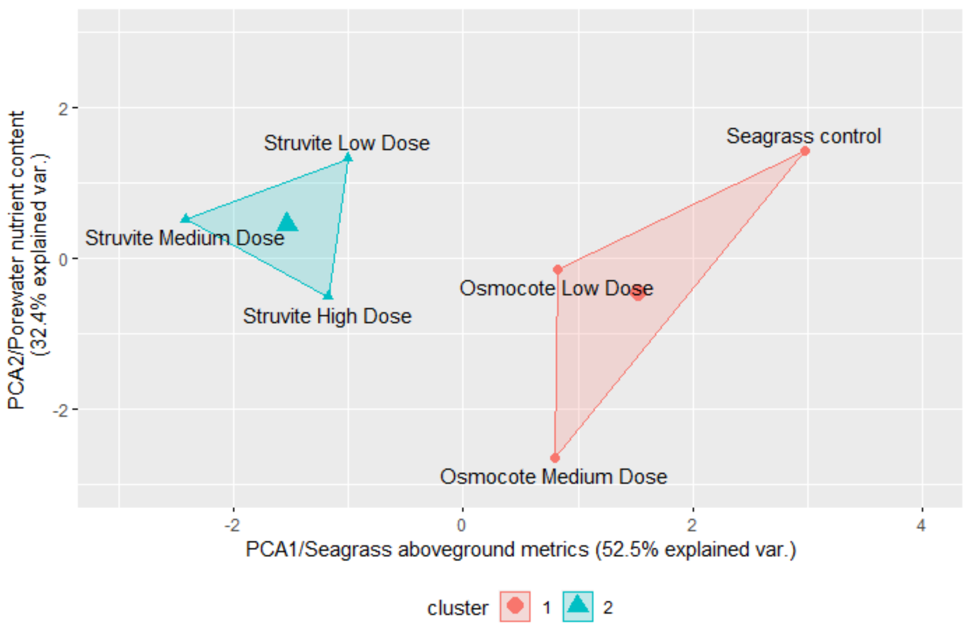

Figure 4. Clustering results of treatment methods from the second experiment. No overlapping clusters were formed, indicating a significantly different global effect of struvite onto the water chemistry and plant growth characteristics compared to Osmocote™ treatment or control plot. Seagrass aboveground metrics (shoot count, blade length and aboveground biomass) were heavily corelated (r > 95%, $p < 0.001$) with first principal component which explained 52.5% of the variance in the dataset. Porewater nutrient dynamics such as TDN and TDP were most corelated ($p < 0.05$) and contributing to second principal component which explained 32.4% of the variance in the dataset. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite).

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Table S-1. Two-way linear mixed effects test results for shoot count and TDP from the single dose experiment/Experiment #1. Factors include treatment, date sampled, and the interaction between these factors.

	Variable					
	Shoot			TDP		
Source	----------count----------			----------mg L^{-1}----------		
Parameter	DF	F statistic	P value	DF	F statistic	P value
Treatment	2	35.91	< 0.0001	5	246.7	< 0.0001
Date	7	10.07	< 0.0001	2	19.50	< 0.0001
Treatment x Date	14	27.95	< 0.0001	10	2.201	0.0328

Table S-2. Two-way linear mixed effects test results for seagrass metrics and porewater nutrients from the multi-dose experiment/Experiment #2. Factors include treatment, date sampled, and the interaction between these factors.

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	Variable											
	Shoot			Length			TDN			TDP		
Source	----------count----------			----------cm----------			----------mg L^{-1}----------					
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	5	0.5703	0.7219	5	13.57	< 0.0001	8	22.37	< 0.0001	8	42.30	< 0.0001
Date	6	18.83	< 0.0001	3	83.75	< 0.0001	2	1.820	0.1686	3	128.2	< 0.0001
Type x Date	30	1.683	0.0289	15	2.118	0.0265	16	1.106	0.3636	24	6.341	< 0.0001

Table S-3. Linear mixed effects test table for biomass taken at the end of the multi-dose experiment/Experiment #2.

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Source	Variable					
	Aboveground Biomass			Belowground Biomass		
	----------g----------					
Parameter	DF	F statistic	P value	DF	F statistic	P value
Type	4	5.231	0.0077	4	4.560	0.0131

Table S-4. Linear mixed effects test table for tissue nutrients (percent weight) taken at the end of the multi-dose experiment/Experiment #2.

	Variable											
	Aboveground %TN			Aboveground %TP			Belowground %TN			Belowground %TP		
Source	----------Percent----------											
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	4	0.784	0.560	3	0.583	0.639	4	3.072	0.049	4	0.539	0.709

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Table S-5. Linear mixed effects test table for tissue nutrient mass (biomass x % nutrients) taken at the end of the multi-dose experiment/Experiment #2. Combined weights present the aboveground TN and TP weights were removed for the analysis (n= 2 for Osmocote^{TM} treatments, unfertilized seagrass removed). Belowground weights for both TN and TP did not have combined samples.

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Source	Variable											
	Aboveground TN Mass			Aboveground TP Mass			Belowground TN Mass			Belowground TP Mass		
	----------g----------											
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	3	1.619	0.2370	3	2.524	0.1070	4	3.276	0.0406	4	3.705	0.0273

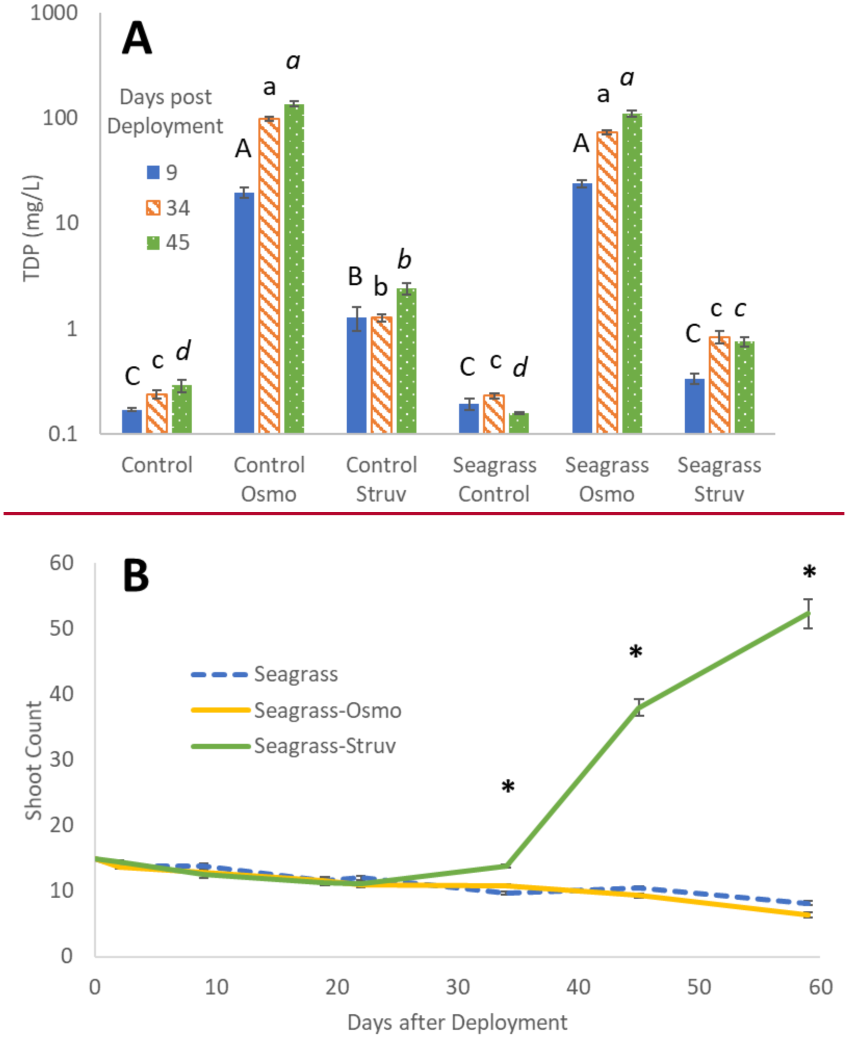
A
TDP (mg/L)
1000
100
10
1
0.1
Days post Deployment
9
34
45
C c d
A a a
B b b
C c d
A a a
C c c
Control
Control Osmo
Control Struv
Seagrass Control
Seagrass Osmo
Seagrass Struv
B
Shoot Count
60
50
40
30
20
10
0
Seagrass
Seagrass-Osmo
Seagrass-Struv
*
*
*
0
10
20
30
40
50
60
Days after Deployment

Table S-6. Mean sediment TC/TN values taken at the end of the multi-dose experiment/Experiment #2. The treatments were labelled control (unfertilized, unplanted plots), Osmo (unplanted fertilized with Osmocote), Struv (unplanted fertilized with struvite), S-Control (unfertilized seagrass plots), S-Osmo-Lo and S-Osmo-Med (planted plots fertilized with Osmocote), S-Struv-Lo, S-Struv-Med, and S-Struv-Hi (planted plots fertilized with struvite).

	Variable			
	TC		TN	
	Mean	SE	Mean	SE
Treatment	----------mg/kg---------			
Control	52.55^{NS}	2.22	2.058^{NS}	0.009
Control-Osmo	57.55^{NS}	2.24	2.073^{NS}	0.015

Control-Struv	53.53^{NS}	6.73	2.036^{NS}	0.016
Seagrass	52.09^{NS}	4.33	2.020^{NS}	0.032
Seagrass-Osmo-Lo	50.56^{NS}	3.28	2.0259^{NS}	0.005
Seagrass-Osmo-Med	49.46^{NS}	3.56	2.053^{NS}	0.027
Seagrass-Struv-Lo	52.02^{NS}	6.17	2.057^{NS}	0.027
Seagrass-Struv-Med	48.70^{NS}	5.02	2.023^{NS}	0.026
Seagrass-Struv-Hi	58.16^{NS}	5.63	2.096^{NS}	0.02

Table S-7. Mixed effects test table for sediment TC/TN taken at the end of the multi-dose experiment/Experiment #2.

	Variable					
-	TC			TN		
Source	---mg/kg---					
Parameter	DF	F statistic	P value	DF	F statistic	P value
Treatment	8	0.5028	0.8435	8	1.376	0.2514

Table S-8.~~Figure 1. Porewater total dissolved phosphorus (TDP) (A), and seagrass shoot count (B) taken during the first mesocosm experiment. The treatments were labelled Seagrass (for the unfertilized seagrass plots), Seagrass-Osmocote™ (for planted plots fertilized with Osmocote™), and Seagrass-Struv (planted plots fertilized with struvite). The asterisks designate significant differences between treatments for the same sample dates. Points represent the mean of six replicates (± SE).~~

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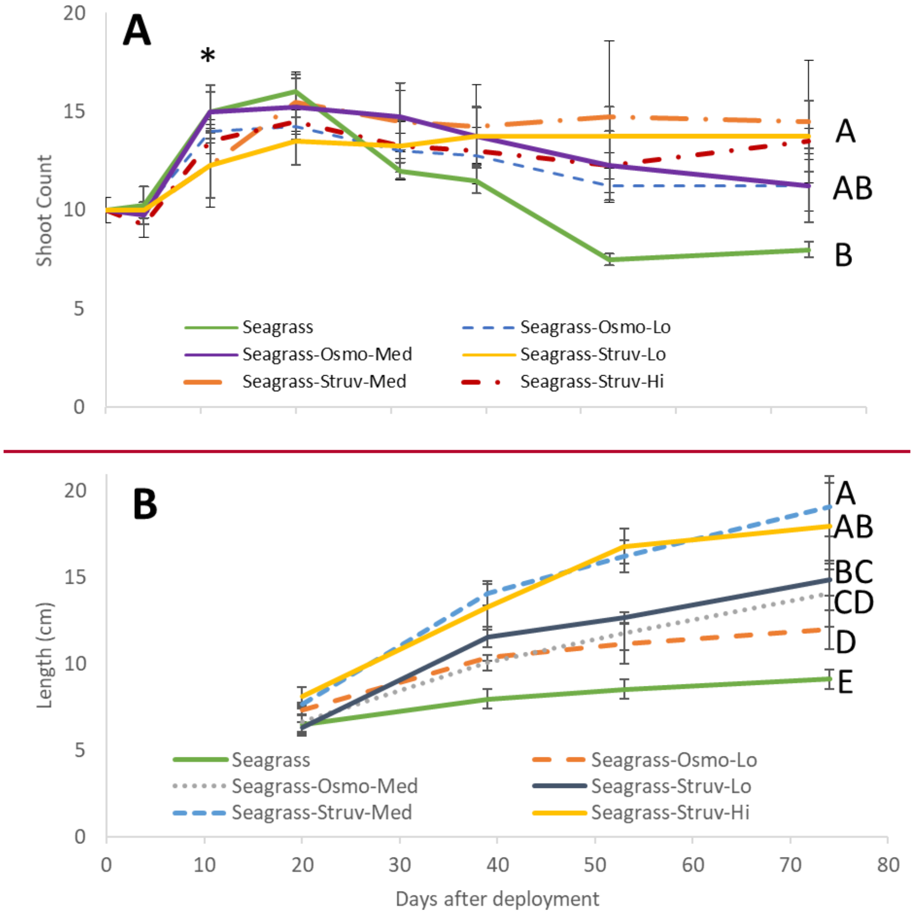

Figure 2. ~~Shoot count (A) and blade length (B) from the second mesocosm experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). *: Five shoots were added to each plot to match the first/single dose experiment. Letters designate significant differences between treatments for the same~~

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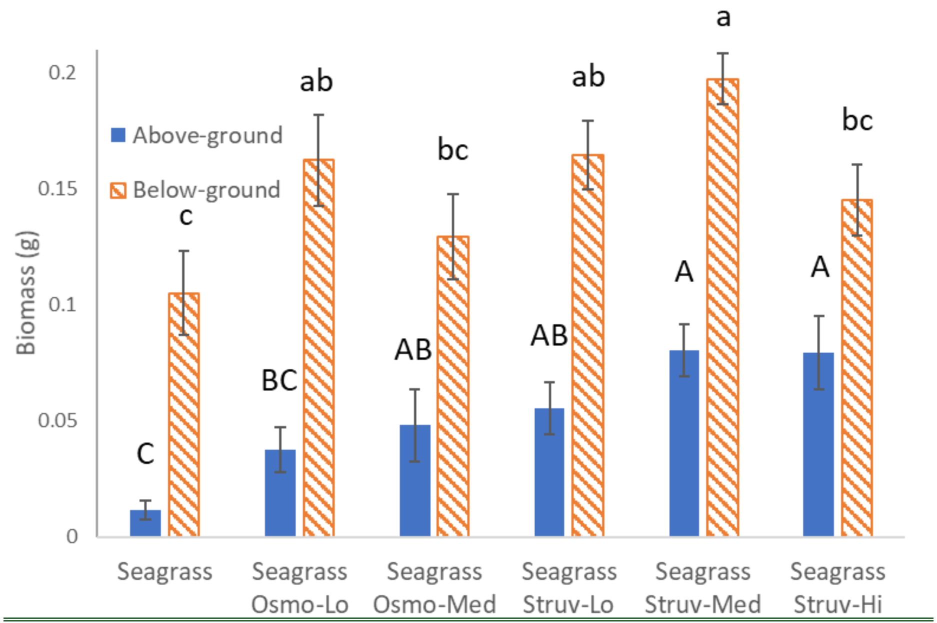
~~sample dates. Points represent the mean of four replicates (± SE), except for above-ground biomass, which had two to four replicates.~~



~~Figure 3. Above and belowground biomass from the second mesocosm experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Letters designate significant differences between treatments for the same sample dates. Points represent the mean of four replicates (± SE), except for above-ground biomass, which had two to four replicates.~~

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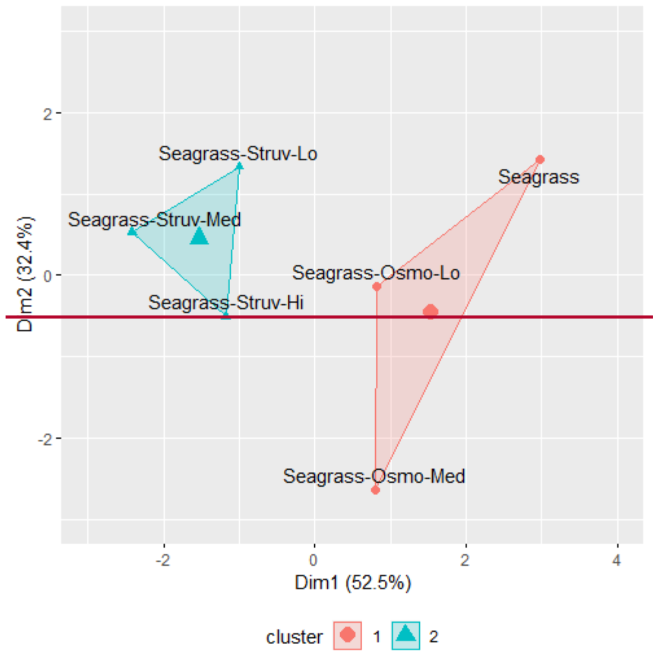

~~Figure 4. Clustering results of treatment methods from the second experiment. No overlapping clusters were formed, indicating a significantly different global effect of struvite onto the water chemistry and plant growth characteristics compared to Osmocote^{TM} treatment or control plot. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote^{TM}), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite).~~

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~~Table S-1.~~ Mean porewater nutrient measurements from the second experiment. The treatments were labelled Control (unfertilized, unplanted plots), Control-Osmo (unplanted fertilized with Osmocote^{TM}), Control-Struv (unplanted fertilized with struvite), Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote^{TM}), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Only TDP was analyzed for Day 31, therefore TDN values at that date are designated "NA." Letters within biomass type represent a significantly different mean based on linear mixed model analyses (NS= not significant).

		Variable			
		TDN		TDP	
		Mean	SE	Mean	SE
Days after Deployment	Treatment	----------mg-/L~~-1~~----------------------			
6	Control	1.51^{NS}	0.38	0.128^{D}	0.004
	Control-Osmo	12.03^{NS}	5.16	9.640^{AB}	3.685
	Control-Struv	4.35^{NS}	1.52	2.093^{C}	0.276
	Seagrass	1.41^{NS}	0.45	0.182^{D}	0.021
	Seagrass-Osmo-Lo	7.89^{NS}	4.34	4.750^{BC}	1.169
	Seagrass-Osmo-Med	26.8^{NS}	7.53	17.68^{A}	6.738
	Seagrass-Struv-Lo	4.33^{NS}	1.60	2.795^{C}	0.962
	Seagrass-Struv-Med	3.32^{NS}	0.88	1.943^{C}	0.211
	Seagrass-Struv-Hi	4.79^{NS}	0.86	2.620^{C}	0.351
20	Control	1.10^{NS}	0.16	0.133^{C}	0.010
	Control-Osmo	19.8^{NS}	2.14	0.508^{A}	0.102
	Control-Struv	5.59^{NS}	1.91	0.303^{ABC}	0.138
	Seagrass	0.78^{NS}	0.14	0.134^{C}	0.016
	Seagrass-Osmo-Lo	6.20^{NS}	2.33	0.289^{ABC}	0.094
	Seagrass-Osmo-Med	12.02^{NS}	0.82	0.472^{AB}	0.279

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	Seagrass-Struv-Lo	2.47^{NS}	0.42	0.272^{ABC}	0.107
	Seagrass-Struv-Med	2.58^{NS}	0.80	0.187^{BC}	0.021
	Seagrass-Struv-Hi	6.81^{NS}	1.73	0.322^{ABC}	0.113

Table S.8. Continued.

	Variable				
		TDN		TDP	
		Mean	SE	Mean	SE
Days after Deployment	Treatment	----------mg/L----------			
31	Control	NA	NA	0.110^{D}	0.009
	Control-Osmo	NA	NA	2.455^{BC}	0.560
	Control-Struv	NA	NA	2.923^{ABC}	0.966
	Seagrass	NA	NA	0.114^{D}	0.016
	Seagrass-Osmo-Lo	NA	NA	1.47^{C}	0.375
	Seagrass-Osmo-Med	NA	NA	9.733^{AB}	6.667
	Seagrass-Struv-Lo	NA	NA	0.163^{D}	0.038
	Seagrass-Struv-Med	NA	NA	1.353^{C}	0.166

~~Table S-1.~~
~~Continued.~~

	~~Variable~~				
		~~TDN~~		~~TDP~~	
		~~Mean~~	~~SE~~	~~Mean~~	~~SE~~
~~Days after Deployment~~	~~Treatment~~	~~----------mg L^{-1}----------~~			
-	~~Seagrass-Struv-Hi~~	~~NA~~	~~NA~~	~~8.220^{A}~~	~~3.634~~
74	Control	1.97^{NS}	0.19	0.085^{C}	0.007
	Control-Osmo	12.2^{NS}	7.54	0.488^{A}	0.135
	Control-Struv	7.75^{NS}	2.00	0.159^{BC}	0.054
	Seagrass	1.68^{NS}	0.15	0.084^{C}	0.021
	Seagrass-Osmo-Lo	5.39^{NS}	0.54	0.261^{AB}	0.067
	Seagrass-Osmo-Med	17.3^{NS}	4.98	0.551^{A}	0.105
	Seagrass-Struv-Lo	4.04^{NS}	1.23	0.162^{BC}	0.064
	Seagrass-Struv-Med	4.74^{NS}	1.03	0.143^{BC}	0.023
	Seagrass-Struv-Hi	9.32^{NS}	1.62	0.156^{BC}	0.017

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Table S-~~2~~9. Mean percent tissue nutrient content and ratio taken at the end of the second experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote^{TM}), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Combined weights present the aboveground TN and TP weights were removed for the analysis (n= 2 for Osmocote^{TM} treatments, unfertilized seagrass removed). Belowground weights for both TN and TP did not have combined samples. Letters within biomass type represent a significantly different mean based on linear mixed model analyses (NS= not significant).

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		TN		TP		TN:TP	
		Mean	SE	Mean	SE	Mean	SE
Biomass Type	Treatment	----------Percent---------				Wt/Wt Ratio	
Above-ground	Seagrass	1.90^{NS}	NA	NA	NA	NA	NA
	Seagrass-Osmo-Lo	2.14^{NS}	0.11	0.258^{NS}	0.016	8.33^{NS}	0.57
	Seagrass-Osmo-Med	2.08^{NS}	0.11	0.249^{NS}	0.017	8.47^{NS}	0.88
	Seagrass-Struv-Lo	2.34^{NS}	0.23	0.236^{NS}	0.007	10.01^{NS}	1.20
	Seagrass-Struv-Med	2.31^{NS}	0.14	0.246^{NS}	0.010	9.38^{NS}	0.29

	Seagrass-Struv-Hi	NA	NA	NA	NA	NA	NA
Below-ground	Seagrass	0.65^{AB}	0.06	0.166^{NS}	0.018	3.90^{NS}	0.36
	Seagrass-Osmo-Lo	0.53^{B}	0.07	0.154^{NS}	0.009	3.44^{NS}	0.47
	Seagrass-Osmo-Med	0.84^{A}	0.06	0.179^{NS}	0.008	4.71^{NS}	0.36
	Seagrass-Struv-Lo	0.66^{AB}	0.05	0.168^{NS}	0.011	3.94^{NS}	0.29
	Seagrass-Struv-Med	0.71^{AB}	0.07	0.164^{NS}	0.011	4.32^{NS}	0.45
	Seagrass-Struv-Hi	NA	NA	NA	NA	NA	NA

Figure S-1. Image showing an example of the differences in above and belowground biomass in seagrasses from the first experiment. Observable stunted roots (possibly root burn) are visible in the Osmocote™ treated plots (SP) versus the control (S) and struvite treated plots (SS).

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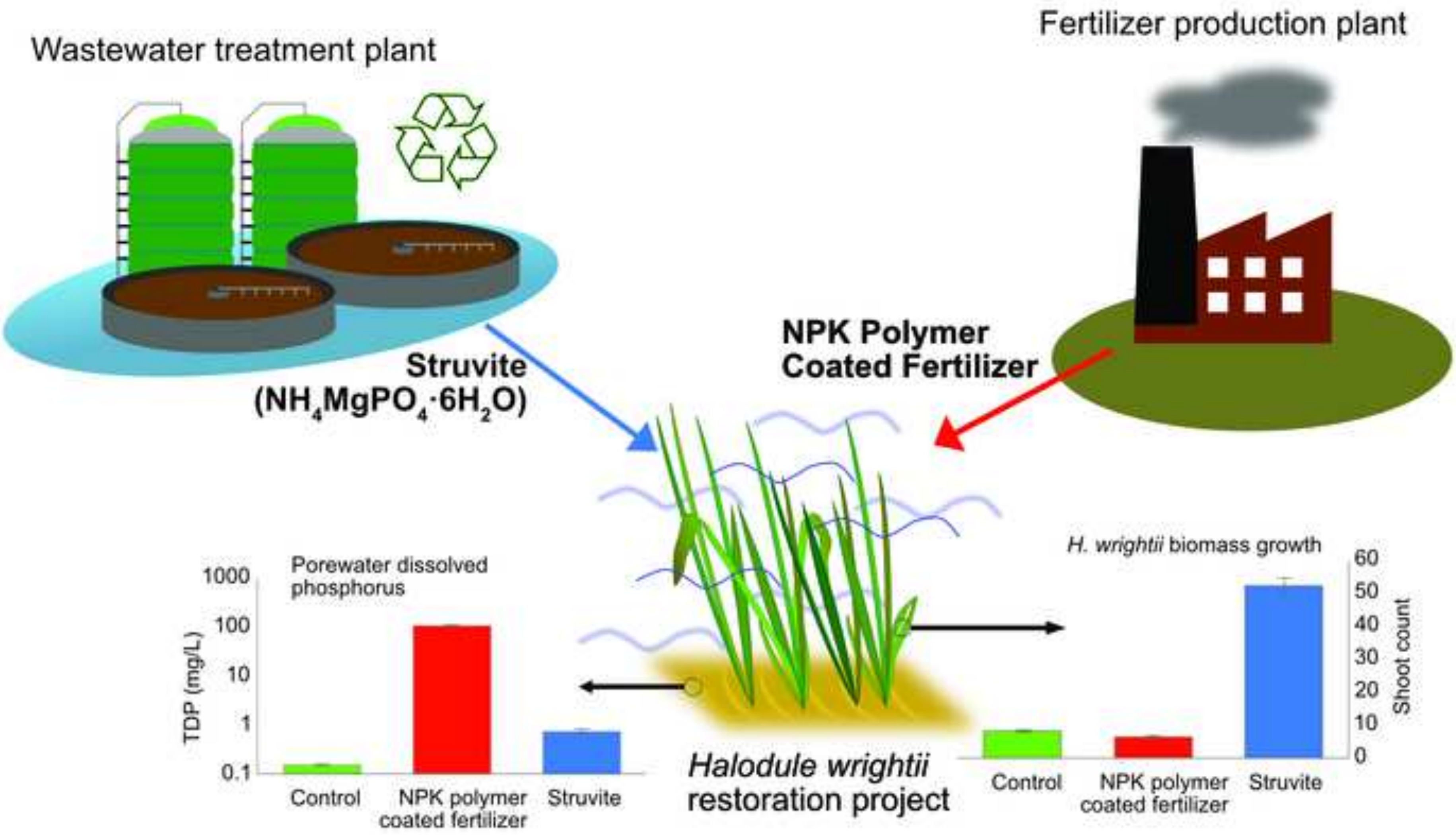
Graphical Abstract



Highlights:

Seagrass restoration is currently expensive and often unsuccessful.

Fertilizers improve restoration but can release excess nutrients.

Osmocote^{TM} and struvite fertilizers were investigated for plant and nutrient metrics.

Struvite produced higher seagrass metrics and released less nutrients.

Abstract

Seagrasses are in decline worldwide, and their restoration is relatively expensive and unsuccessful compared to other coastal systems. Fertilization can improve seagrass growth in restoration but can also release nutrients and pollute the surrounding ecosystem. A slow-release fertilizer may reduce excessive nutrient discharge while still providing resources to the seagrass's rhizosphere. In this study, struvite (magnesium ammonium phosphate), a relatively insoluble, sustainable compound harvested in wastewater treatment plants, was compared to Osmocote^{TM} (14:14:14 Nitrogen: Phosphorus: Potassium, N:P:K), a popular polymer coated controlled release fertilizer commonly used in seagrass restoration. Two experiments compared the effectiveness of both fertilizers in a subtropical flow-through mesocosm setup. In the first experiment, single 0.5 mg of P per g dry weight (DW) doses of Osmocote^{TM} and struvite fertilizers were added to seagrass plots. Seagrass shoot counts were significantly higher in plots fertilized with struvite than both the Osmocote^{TM} and unfertilized controls ($p < 0.0001$). A significant difference in total P concentrations was observed in porewater samples of Osmocote^{TM} vs struvite and controls ($p < 0.0001$), with struvite fertilized plots emitting more than controls ($p \leq 0.0001$), but less than 2% of the total dissolved P (TDP) of Osmocote^{TM} fertilized plots (100+ mg/L versus $x > 5$ mg/L). A subsequent experiment, using smaller doses (0.01 and 0.025 mg of P per gram DW added), also found that the struvite treatments performed better than Osmocote^{TM}, with 16-114% more aboveground biomass (10-60% higher total biomass) while releasing less N and P. These results indicate the relatively rapid dissolution of Osmocote^{TM} may pose problems to restoration efforts, especially in concentrated doses and possibly leading to

seagrass stress. In contrast, struvite may function as a slow-release fertilizer applicable in seagrass and other coastal restoration efforts.

Keywords: *Halodule wrightii*; seagrass; marine restoration; fertilizer; struvite; Osmocote^{TM}; phosphorus

1. Introduction

In many environments, restoration is improved by fertilization, lessening nutrient limitations and improving growth of desired species (Armitage et al., 2011; Balestri & Lardicci, 2014; Fereidooni et al., 2013; Holmes, 2001; Jaquetti et al., 2014; Reed et al., 2007). However, in some environments, fertilizers can have a negative effect on species diversity and in extreme cases may even pollute the surrounding environment (Fonseca et al., 1998; Hill & Heck, 2015; Zedler, 2000). Therefore, consideration of the ecosystem, nutrient needs, and type of fertilizer is important to maximizing the benefits of fertilization approaches while minimizing the environmental impact of fertilizer use.

The ramifications of fertilizer use are especially relevant in coastal seagrass systems, which are both important habitats and currently facing global declines due to human disturbance and climate change (Bayraktarov et al., 2016). Seagrasses are a comparatively difficult and expensive coastal ecosystem to restore, partially due to eutrophication, competition from algae and other nutrient related issues (ibid). However, fertilizers have been consistently found to improve seagrass health and restoration success (Armitage et al., 2011; Kenworthy et al., 2018). Traditionally, both the direct application of controlled release fertilizers (Armitage & Fourqurean, 2016; Fonseca et al., 1998; Peralta et al., 2003; Sheridan et al., 1998) and the deployment of bird roosting stakes (Fonseca et al., 1994; Furman et al., 2019) have positive effects on seagrass biomass, and can accelerate ecosystem succession for seagrass (Bourque &

Fourqurean, 2014; Armitage et al., 2011). However, the use of traditional fertilization techniques in seagrass restoration may result in variable levels of nutrients or over-fertilization (Fonseca et al., 1998; Kenworthy et al., 2018), with consequences for the succession of seagrass species (ibid).

One of the main issues with fertilization in aquatic seagrass systems is that immersion and hydrodynamics can lead to rapid dissolution of fertilizers, increasing short term nutrient availability to the desired plant species, but at the expense of nutrient loss, ecosystem disruption, and pollution (Fonseca et al., 1998; Hill & Heck, 2015; Olsen & Valiela, 2010). For example, Hall et al. (2006) had to replace buried fertilizer pellets every three to four months in a macrophyte restoration effort, while Herbert and Fourqurean (2008) found that bird stakes (bird roosting structures that promote feces accumulation, Fonseca et al., 1994; Furman et al., 2019) can overfertilize seagrass sites, disrupting succession and increasing epiphytic biomass. These drawbacks are due either to the fertilizers being adapted for terrestrial applications, releasing nutrients too rapidly after flushing with water, or in the case of bird stakes, due to variable rates of feces deposition combined with diffusion of nutrients in the water during precipitation and settling (Hill & Heck, 2015). Applying multiple doses of traditional mineral fertilizers (Ferdie & Fourqurean, 2004; Hall et al., 2006; Olsen & Valiela, 2010) or monitoring bird stake treated beds for symptoms of excess fertilization (Kenworthy et al., 2018) also incurs a significant financial and labor cost. Thus, a slower dissolving fertilizer that resists leaching may reduce overfertilization and labor expenses while still providing benefits toward seagrass growth and survival.

Struvite (magnesium ammonium phosphate, or $MgNH_4PO_4 \cdot 6H_2O$) is a by-product of wastewater treatment that is harvested in separated, side-stream sludge management processes (Ghosh et al., 2019). Struvite is poorly soluble in water, but releases P more rapidly in the presence of organic acids exuded from roots, making it a potentially ideal fertilizer for direct plant uptake (Cabeza et al., 2011; Robles-Aguilar et al., 2019). Past studies have supported both high performance of struvite for terrestrial plant applications as well as its resistance to flushing (Lee et al., 2009; Rahman et al., 2014).

While the utilization of struvite in aquatic systems appears very promising, to date there is an absence of studies investigating this fertilizer in marine restoration projects, especially in combination with other fertilization techniques. While it has been demonstrated that struvite is poorly soluble fertilizer except when exposed to acidic conditions (Cabeza et al., 2011; Talboys et al., 2016), experiments determining the availability of struvite to submerged aquatic vegetation do not currently exist. Thus, the goals of this study were to 1) assess potential differences in seagrass performance (shoot count, growth, length, and biomass as defined by Arrington, 2008, Herbeck et al., 2014, Rezek et al., 2019, Short & Coles, 2001, and Thomsen et al., 2012) after addition of struvite versus a polymer coated, controlled release fertilizer (Osmocote^{TM}) commonly used in seagrass restoration, and 2) to determine shifts in sediment and porewater nutrients caused by the introduction of the fertilizers in plots with and without seagrass. We hypothesized that seagrass in plots fertilized with struvite would have increased performance compared to plots fertilized with Osmocote^{TM}, and that struvite would be dissolved at a slower rate than Osmocote^{TM} (based on porewater total dissolved nutrients).

2. Materials and Methods

2.1. Site Description and Design

To minimize the variability found in field experiments and more accurately investigate nutrient levels related to fertilization, a mesocosm experiment was conducted at the Whitney Laboratory of Marine Biosciences in St. Augustine, FL. Seawater (filtered through a shelly sand and activated charcoal biofilter) pumped from offshore entered a 6.5 m diameter mesocosm (approximately 1 m deep), to emulate the natural environment. Water flow was constant into the mesocosm. Experiments were based on the methods explained in the propagation guide for *Halodule wrightii* (Biber et al., 2013). Seagrass was collected directly from donor sites off St. Martins Marsh Aquatic Preserve, FL. Shoots were removed from the donor sediment and maintained in cool conditions until they were transplanted into plastic pot containers (10 cm depth), buried in approximately 5 cm of coarse, shell-dominated sand taken from the local St. Augustine area (rinsed to reduce organics and residual nutrients). The sediment used had a mean grain size of 706 microns (not including particles greater than 2 mm).

2.1.1 Mesocosm Conditions

Mesocosm temperature and salinity remained between 27-31 °C and 33-38 parts per thousand respectively during the periods sampled (between 9 am and 3 pm) for both studies. The hydraulic residence time was variable at 0.5-2 days, due to a limited saltwater supply. The mean TDN of surface water was 0.44 ± 0.06 mg N L^{-1}, while the mean TDP was 0.035 ± 0.001 mg P L^{-1} (or 0.029 mg P L^{-1} when excluding a day of low inflow). The level of flow was great enough to prevent significant cross contamination of the plots studied, as well as prevent significant swings in temperature and salinity that could stress the plants.

2.2. Experiments

Two separate experiments were conducted in the summer and fall of 2018. The first 60-day experiment consisted of six different treatment options, including bare sand with or without fertilizers (terrestrial polymer coated fertilizer or struvite) and seagrass with or without fertilizers. A second 70-day experiment was conducted consisting of multiple lower doses of both fertilizers.

2.2.1. Single Dose/First Experiment

For the polymer coated controlled release fertilizer treatment, Osmocote^{TM} 14:14:14 NPK (Scotts Miracle-Gro Company, Marysville, OH, USA) was chosen due to its commercial availability, composition (containing both N and P), and past use in seagrass restoration experiments (Peralta et al., 2003; Sheridan et al., 1998; Tanner & Parham, 2010). Struvite used in the experiment was produced in a pilot scale fluidized bed reactor fed with sludge dewatering liquor. Detailed morphological and elemental characteristics are described elsewhere (Bydałek et al., 2018). Unlike the mostly homogenous struvite, each Osmocote^{TM} prill has a porous outer layer that gradually releases a contained water-soluble nutrient dose through diffusion. The composition of elements is also different between the two compounds; with NH_4^+/NO_3^--N comprising 14% of Osmocote^{TM} versus NH_4^+-N comprising only 6% of struvite (Osmocote^{TM} manufacturer information, Kenworthy & Fonseca, 1992; Rahman et al., 2014). The P composition of both fertilizers is also different, with struvite (13% P as PO_4^{3-}) versus having a higher concentration by weight versus Osmocote^{TM} (6.1% P as P_2O_5) (Osmocote^{TM} manufacturer information, Rahman et al., 2014).

In total, there were 30 plots, with an unplanted, untreated/unfertilized control (labelled control, n= 4), sediment-only treatments (labelled Control-Osmo and Control-Struv, n= 4), and seagrass control and treatments (labelled Seagrass, Seagrass-Osmo, and Seagrass-Struv, n= 6). Nutrient treatments were fertilized by adding the Osmocote^{TM} or struvite equivalent of 3 g of P mixed into approximately 6 kg of sand (equivalent to 0.5 mg P g^{-1} DW sand), which was about half of what was considered "low fertilized" according to Peralta et al. (2003). The dosing was equilibrated to P as tropical seagrass systems are primarily P limited (Brodersen et al., 2017; Gras et al., 2003). In this experiment, serving as pilot study, N concentrations were not equilibrated, however given the actual fertilizer dosages, concentrations were still below the low fertilized treatment in Peralta et al.'s study (0.23 mg N g^{-1} DW sand for struvite and 1.16 mg N g^{-1} DW sand for Osmocote respectively). Each seagrass plot had exactly three individuals, each with five shoots. The first experiment was conducted for 60 days. During this period, the levels of dissolved total P porewater concentrations were excessively high, exceeding 100 mg P L^{-1} in the Osmocote^{TM} treatments and 5 mg P L^{-1} for struvite.

2.2.2. Multi-Dose/Second Experiment

In this second experiment, struvite doses were 0.0125 (low dose struvite or Seagrass-Struv-Lo), 0.025 (medium dose struvite or Seagrass-Struv-Med) and 0.05 mg P g^{-1} DW sand (high dose struvite or Seagrass-Struv-Hi). For Osmocote^{TM}, 0.0125 (low dose Osmocote^{TM} or Seagrass-Osmo-Lo) and 0.025 mg P g^{-1} DW (medium dose

Osmocote^{TM} or Seagrass-Osmo-Med) doses were used. Unplanted, fertilized controls had a 0.0250 mg P g^{-1} DW dose of Osmocote^{TM} (Osmocote^{TM} control or Control-Osmo) and struvite (struvite control or Control-Struv). Unfertilized, unplanted plots were labelled “control” while unfertilized, planted plots were labelled “unfertilized seagrass” or “Seagrass-Control”. There were four replicates for all controls/treatments. A high dose of Osmocote^{TM} was not used due to space limitations in the mesocosm and concerns of overfertilization based on the results of the single dose/first experiment. There were three individuals with five shoots per plot (initially two individuals with the third added 10 days post deployment to match the starting shoot count of the previous experiment).

2.3. Plant and Nutrient Measurements

Seagrass shoot count (seagrass shoots defined as a unit of several leaves or blades according to Short & Coles, 2001), were quantified approximately every 10 days in both experiments. During the second experiment, blade/leaf lengths (substrate to leaf tip according to Arrington, 2008) were also quantified. Surface water was sampled for temperature, salinity, and total dissolved nutrients (Total Dissolved N/TDN, Total Dissolved P/TDP), while porewater was only sampled for total nutrients and (randomly) sulfide presence. Surface and porewater samples were collected using a syringe sampler fashioned out of a 60 mL syringe attached to a plastic tube and 1 mL serological pipette with an attached air stone. The samples were filtered through a 0.45 μm filter (Whatman, Maidstone, United Kingdom), preserved with sulfuric acid to a pH < 2, and stored at 4 ºC until analysis in the Wetland Biogeochemistry Laboratory (USEPA, 1974, 1993). Porewater was also tested for the presence of sulfide (Calleja et al., 2007; Carlson et al., 1994) using a Hach test kit (product number 2537800). No measurable sulfide was found in any plots sampled (detection limit 0.1 mg L^{-1}). DOC and TDN samples were analyzed on a Shimadzu TOC-L analyzer fitted with a N module (Shimadzu Scientific Instruments, Durham, NC, USA) according to EPA method 415.1 for TOC and ASTM D 8083 for total nitrogen (TN) (ASTM International, 2016; Nevins et al., 2020; USEPA, 1974). TDP was digested with persulfate in an autoclave and analyzed via a Shimadzu UV-1800 spectrophotometer (Shimazdu Corporation, Kyoto, Japan) using EPA method 365.1 (Irick et al., 2015; USEPA, 1993).

At the end of the experiment, plant biomass and sediment were destructively sampled. Plants were rinsed to clean off sediments, and promptly frozen. In the lab, tissue samples were cleaned of epiphytes and rinsed with de-ionized water. Plant tissue and sediment samples were dried for 72 hours at 65 ºC and ground using a ball mill. Sediment was analyzed for total carbon (TC), and nitrogen (TN), while tissue was analyzed for TC, TN, and phosphorus (TP). Bulk sediment TC/TN were run on an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA) (Nevins et al., 2020). Tissue TP was determined by ashing the sample followed by dissolution with 6 M HCL (following Andersen, 1976) and analysis for soluble P using a Shimadzu UV-1800 spectrophotometer (Shimazdu Corporation, Kyoto, Japan) (Liao et al., 2019; USEPA, 1993). Due to low and variable weights found after drying seagrass samples, plant dry biomass was calculated using a 10% wet weight conversion used for *H. wrightii* and *Thalassia testudinum* in Heck et al., (2015) and outlined in Short & Coles, (2001). A sediment particle analysis was also conducted to determine the distribution of particle sizes and possible changes over time. These samples were analyzed by the Soil and Water Sciences Environmental Pedology and Land Use

Laboratory using laser diffraction (LD) with a Beckman Coulter LS-13320 multi-wave particle size analyzer (Beckman Coulter Diagnostics, Brea, CA, USA).

2.4. Statistical Analyses

Differences in seagrass metrics (shoot count and shoot length) and porewater nutrients for both experiments were calculated using a linear mixed model, followed by a post hoc multiple comparison significant (Fisher's Least Significant Difference test). Factors included the treatment type, date, and the interaction between treatment and date. A linear mixed model analysis was also conducted on sediment and biomass measurements from the second experiment, testing the effect of treatment type. The tests were run using JMP 15.2.1 (SAS Software, Cary, NC, USA) with significance set to $\alpha = 0.05$. To determine the fit of the model predictions to the measured data, residuals and qq-plots were visually inspected and data was log transformed as necessary (shoot counts, shoot lengths, and total dissolved nutrients). To differentiate between the effects of fertilization methods, K-means clustering was applied to classify all observations in the multi-dose/second experiment. K-means were computed using the `kmeans` function in R (version R-4.0.2.). Given the number of observations (n= 6) the data was predefined into two clusters (`centers= 2`). Prior to the analysis, the data was standardized using the `scale` function (each element is subtracted by the mean value of the vector and divided by standard deviation of the vector). The results were visualized using the `fviz_cluster` function (factoextra package) based on function's encoded principal component analysis (PCA) (Kassambara & Mundt, 2017).

3. Results

3.1. Single Dose/First Experiment

3.1.1 Plant Metrics

Increases in shoot counts occurred one month after transplantation for the struvite treatment. However, this was not the case with the unfertilized control or the Seagrass-Osmo treatment, which both slowly declined on average. At the end of the first experiment, mean shoot counts ranged from 6.33 ± 0.87 shoots in the Seagrass-Osmo treatment to 52.33 ± 5.49 shoots in the Seagrass-Struv treatment (Figure 1). Seagrasses in struvite fertilized plots had significantly higher shoot counts than the seagrass control and Osmocote treatment ($p < 0.01$). More specifically, the Seagrass-Struv treatment had a significantly higher shoot count in mid-July, just one month after planting ($p < 0.05$), becoming greater over the next month (by end of the study $p < 0.001$). By the end of the study, the unfertilized seagrass also had a significantly higher number of shoots than the Seagrass-Osmo treatment ($t = 2.56$, $p < 0.05$).

3.1.2 Water Chemistry

The TDP levels were significantly higher in the Seagrass-Osmo plots than the unfertilized controls and Seagrass-Struv treatments ($p < 0.0001$, table S1). By the end of the study, the average TDP concentration for the Seagrass-Osmo porewater plots was 136.09 ± 15.71 mg P L^{-1} for the unplanted plots (Control-Osmo) and 109.53 ± 19.96 mg P L^{-1} for the planted plots (Seagrass-Osmo), more over ten times higher than the struvite plots, which was 2.43 ± 0.61 mg P L^{-1} in the unplanted plots and 0.76 ± 0.19 mg P L^{-1} in the Seagrass-Struv plots. Porewater TDP in the Control-Struv treatment was significantly higher than the control, unfertilized seagrass, and the Seagrass-Struv treatments ($p < 0.001$), indicating that significant uptake of TDP by seagrasses likely occurred. There were no significant differences in TDP between the unplanted and planted Seagrass-Osmo plots, overall or during any specific sampling date.

3.2. Multi-Dose/Second Experiment

3.2.1. Plant Metrics

At the end of the second experiment, the average seagrass shoot counts ranged from 8.00 ± 0.41 shoots in the Seagrass-Control to 14.50 ± 3.10 shoots in the Seagrass-Struv-Med treatment (Figure 2). There was relatively less growth in the second experiment versus the first/single dose experiment, however the effects of date and its interaction with the treatment type were still significant for shoot count (Table S-2). After 53 days, seagrass shoot count started showing signs of treatment effect in comparison to control seagrass plot which showed significant shoot count declines ($p < 0.05$) in comparison to the rest of the fertilized seagrass plots. By the end of the experiment (74 days) only the Seagrass-Struv-Med treated seagrass plots maintained plant density (14.50 ± 3.10 shoots) close to the original coverage of 15 shoots per plot indicating high transplantation survival rate. At the conclusion of the study, only the struvite fertilized plots were statistically higher in shoot count than unfertilized plots.

The effects of both treatment and date were significant for blade length (Table S-2). All fertilized treatments became significantly greater in length than the Seagrass-Control after 39 days post deployment (Figure 2). The average seagrass blade length ranged from 9.1 ± 1.02 cm in the unfertilized seagrass to 19.1 ± 1.74 cm in the medium dose struvite by the end of the experiment. The highest increase in blade length was observed in struvite treatments. The Seagrass-Struv-Med treatment showed a significantly ($p < 0.005$) higher blade growth than the Seagrass-Osmo-Lo/Med treatments.

The mean aboveground biomass ranged from 0.012 ± 0.004 g DW in the Seagrass-Control to 0.080 ± 0.011 g DW in the Seagrass-Struv-Hi treatment (Figure 3), with the effect of treatment type being significant (Table S-3). All fertilized plots had significantly higher aboveground biomass than the control, except for the Seagrass-Osmo-Lo treatment ($p < 0.05$). There was a marginal significance found for the Med and Hi struvite doses having higher aboveground biomass than the Seagrass-Osmo-Med ($p < 0.08$). Belowground biomass ranged from 0.11 ± 0.02 g DW in the Seagrass-Control to 0.20 ± 0.01 g DW in the Seagrass-Struv-Med treatment (Figure 3). The belowground biomass of control plots was significantly lower compared to all fertilized plots except for the Seagrass-Osmo-Med (Table S-3). Additionally, the Seagrass-Struv-Med dose had significantly higher belowground biomass compared to the Seagrass-Osmo-Med and Seagrass-Struv-Hi doses ($p < 0.05$). Aboveground tissue %TN ranged from 1.9% in the unfertilized seagrass (one sample) to 2.34 ± 0.23% in the Seagrass-Struv-Lo treatment, while tissue %TP ranged from 0.236 ± 0.007% in the Seagrass-Struv-Lo treatment to 0.258 ± 0.016% in the Seagrass-Osmo-Lo treatment (Table S-7). There was no significant effect of treatment on aboveground %TN or %TP (Table 4). The mean aboveground N:P ratios ranged between 8.3 ± 0.57 for the Seagrass-Osmo-Lo and 10.0 ± 1.20 for Seagrass-Struv-Lo treatment. The N:P ratio and the mean aboveground TN and TP weights in the seagrasses (calculated by multiplying the biomass with the tissue %TN or %TP) yielded no significant differences (Tables S-4 and 7). Belowground tissue %TN ranged from 0.53 ± 0.07% for the Seagrass-Osmo-Lo treatment to 0.84 ± 0.06% in the Seagrass-Osmo-Med treatment, while tissue %TP ranged from 0.154 ± 0.009% for the Seagrass-Osmo-Lo treatment to 0.179 ± 0.008%

for the Seagrass-Osmo-Med treatment. The effect of treatment type was significant for belowground %TN (Table 4), with the Seagrass-Osmo-Med being significantly higher than the Seagrass-Osmo-Lo ($p< 0.05$). No effects were significant for belowground %TP. The mean belowground N:P ratio ranged from 3.4 ± 0.47 for the Seagrass-Osmo-Lo and 4.7 ± 0.36 for the Seagrass-Osmo-Med treatment. The effect of treatment type was significant for both the belowground mass of TN and TP (% total nutrient x biomass, Table S-5).

3.2.2. Water, Tissue, and Sediment Chemistry

Nutrient dynamics in porewater differ significantly between the fertilizer types indicating different dissolution kinetics and plant and substrate interaction. Unfertilized control plots (planted and unplanted) showed variable TDN concentrations throughout the experiment however, never surpassing 2 mg TDN L^{-1}. Background porewater TDP content in observed controls varied within 0.05-0.15 mg TDP L^{-1}. The biggest nutrient release was observed at plots fertilized with Osmocote with peak nutrient concentrations occurring at 6th day of experiment reaching 26.8 ± 7.53 mg TDN L^{-1} and 17.68 ± 6.74 mg TDP L^{-1} for medium Osmocote dose. TDP dynamics in struvite seagrass treatments were highly variable throughout the time and showed alternating pulses of TDP release. However, by the end of the experiment porewater TDP content in struvite fertilized plots was 2-3 times lower than in respective Osmocote treatments. DOC measured at the end of the study was between 12.26 ± 0.67 mg DOC L^{-1} for Seagrass-Struv-Lo and 14.71 ± 1.23 mg DOC L^{-1} for Seagrass-Osmo-Lo.

The average TC content of sediment ranged from 48.7 ± 5.02 g C kg^{-1} in the medium dose struvite to 58.2 ± 5.63 g C kg^{-1} in the Seagrass-Struv-Hi, while the average TN content ranged from 2.02 ± 0.032 g N kg^{-1} in the Seagrass-Control to 2.10 ± 0.020 g kg^{-1} in the Seagrass-Struv-Hi treatment (Table S-6). There were no significant differences in the TC or TN contents between treatments (Table S-7).

Porewater nutrients and seagrass metrics were used to further assess the global effect of fertilization dose and method based on multivariate analysis. K-means clustering detected two separate groups. The struvite treatment was clearly distinguished from the Osmocote^{TM} treatment and control plot, occupying separated, non-overlapping clusters on the PCA plane (Figure 4), reinforcing the significant effects of struvite on seagrass and its surrounding environment.

4. Discussion

4.1. Factors in Seagrass Performance

Fertilizer application improved seagrass metrics compared to the unfertilized control in all but the Seagrass-Osmo treatment of the first experiment. This included average shoot count (more than six times higher vs the control at the end of the first experiment, and up to 81% at the end of the second experiment), length (up to 110% at the end of the second experiment, Figure 2), and biomass (up to 138% at the end of the second experiment, Figure 3). In general, these results support past findings examining the effects of fertilizer in the restoration of seagrass ecosystems (Armitage et al., 2011; Kenworthy et al., 2018). Additionally, the results of this study found that compared to equivalent P dosages with Osmocote, fertilization using struvite resulted in higher average seagrass shoot count (more than eight times higher by the end of the first experiment, and 29% at the end of the second experiment), length (up to 36% at the end of the second experiment, Figure 2), and biomass (up to 60% higher total biomass at the end of the second experiment, Figure 3). The significant multivariate improvements in plant metrics in both experiments are promising towards the use of struvite as a fertilizer to rapidly establish seagrass species in future restoration efforts.

In addition to improving seagrass metrics, struvite consistently released less nutrients than Osmocote^{TM}. Porewater TDN was excessive in the Osmocote^{TM} treatment in the first experiment (> 100 mg/L). In the second experiment, TDN in struvite treated plots was as low as 12% of Osmocote^{TM} treated plots (Table S-8). Porewater TDP in equivalent struvite doses was less than 2% TDP of Osmocote^{TM} in the first experiment (Figure 1), and as low as 10% P of Osmocote^{TM} in equivalent struvite doses in the second experiment (Table S-8). The speed of nutrient release by Osmocote^{TM} was so high, that it may have contributed to the decreased performance of the Osmocote^{TM} treatments through excessive N levels, as evidenced by roots that appeared stunted from possible root burn (observed in the first experiment, Figure S-1), commonly associated with N exposure (NC State, 2018; Schönau & Herbert, 1983).

The possible root burn in Osmocote^{TM} treated seagrass may be the result of nitrate (Peralta et al., 2003; Statton et al., 2014) or ammonia (van der Heide et al., 2008) fractions in the fertilizer. However, previous seagrass (*Zostera marina*) mesocosm studies have detected increased seagrass metrics following Osmocote^{TM} fertilization. For example, *Zostera marina* plants were found to have increased shoot counts after one month of Osmocote^{TM} 14:14:14 NPK fertilizer exposure compared to unfertilized plots (Wang et al., 2020). Similarly, another study found significant differences in shoot length in *Z. marina* over a period of two months when exposed to fertilizer doses higher than those used in this study (Peralta et al., 2003). In these cases, it should be noted that *Z. marina* exhibited a “remarkable tolerance” of N and P fertilization, and many species of seagrass may not be as flexible regarding higher levels of nutrient exposure.

Another factor affecting the difference between struvite and Osmocote^{TM} could be the balance of N versus P. In the second experiment, the aboveground tissue N:P ratios (8.3 ± 0.57 to 10.0 ± 1.20, Table S-9) consistently exceeded the traditionally accepted threshold for a balanced nutrient supply for seagrasses (14 weight N:P ratio calculated from the 30:1 molar N:P ratio as provided by Atkinson & Smith, [1983]). A study of *H. wrightii* found that in a natural system (Florida Bay) the molar N:P was over 20, while in a fertilized scenario (using bird roosting stakes) the ratio was approximately 13 (Powell et al., 1989). Thus, the authors argued that *H. wrightii* was P limited in a natural setting, and N limited when fertilized. Another study in Florida Bay found that *H. wrightii* was “released” from P limitation at tissue N:P weight ratios between 9.7 and 21 (Armitage et al., 2011). Generally, the *H. wrightii* in all fertilized plots did not appear to be strongly limited by a specific nutrient, exceeding the 1.8% TN/ 0.2% TP tissue nutrient requirement defined by Duarte (1990). The exception to this may have been the control, which was closer to N limitation than all plots with a 1.9% TN tissue content, although this conclusion is tenuous because only one replicate was able to be analyzed due to a lack of biomass.

The lack of significant differences in tissue nutrient content between fertilized and non-fertilized treatments may be due to delays in nutrient response by the plants. For example, one study found that it took *Thalassia testudinum* four months to acquire elevated N levels after fertilizer exposure, while elevated P levels in plants took up to 14 months to develop (Ferdie & Fourqurean, 2004). While *H. wrightii* is a faster growing species, and higher growth was demonstrated in fertilized vs non-fertilized plots, the limited experiment duration may not have fully captured long-term increases in tissue content. However, significant differences in belowground nutrient content (i.e. medium dose struvite vs. non-fertilized control, Table S-4) and tissue nutrient weight (Table S-5) indicate uptake of nutrients by the seagrass.

Furthermore, the size of the mesocosm plots may have been a factor in the high porewater nutrient levels by preventing lateral flow of porewater and limiting diffusion. The current flow and increased sediment depth may dilute porewater, increase diffusion, and reduce the effectiveness of fertilizers in a natural environment, requiring more fertilizer for field studies. This potential problem may be partially compensated by the relatively large grain size of the shelly sand used in the study, compared to the often silty sand found in seagrass systems (a property produced by seagrass beds as discussed in Folmer et al., 2012). The lack of sulfide present in the experiment also indicates a higher redox potential that is likely not present in field experiments.

This study demonstrated that struvite and Osmocote^{TM} both released N and P for at least two months (Table S-8). Based on longer studies, it is expected that Osmocote^{TM} would provide N and P for 4-6 months (Hall et al., 2006; Olsen and Valiela, 2010). Struvite may be able to provide nutrients for longer periods, indicated by its slower release rate. After the second experiment, selected fertilized plots were moved to another mesocosm and left submerged. A year after the experiment was deployed, the only evidence found of the Osmocote^{TM} fertilizer were the outer membranes of the prills, whereas struvite granules were still found in the mesocosm plots, indicating a potential continued release of nutrients. Thus, while the effects of struvite were only measured for up to nine weeks, the presence of struvite after this extended period indicates that struvite could be effective throughout a whole growing season or longer. The ability of struvite to produce higher seagrass metrics while emitting less nutrients (indicating a more sustained release of nutrients over a longer period of time) is promising toward the future applications of struvite in future coastal restoration efforts.

4.2. Field Applications of the Study

The controlled environment of the mesocosm study allowed tests to be done with minimal interference from the confounding variables of a field study. However, several external factors may still have affected the results of the two experiments. The first experiment was conducted at the peak of the seagrass growing season (June through August), whereas the second experiment occurred during the end of the season (August through October, with the season typically ending in September; Choice et al., 2014). The later date of deployment could help explain why differences between shoot counts were not as apparent in the second experiment. Based on the declining seagrass performance above the medium/0.025 mg P g^{-1} DW dose, there may have been even larger differences in the first experiment between struvite and Osmocote^{TM} if the second experiment was begun earlier in the summer.

When considering the broad applicability of the results, it is important to note how close the conditions in the mesocosm were mimicking the natural environment. First, the local sediment substrate was not sterilized and contained a representative microbial population. Similarly, seawater for the mesocosm was only prefiltered to minimize inputs of algae or debris, and largely maintained the natural composition and physiochemistry. The mesocosm environment was sheltered from hydrodynamic disturbance and herbivory which are significant problems in field restoration efforts (Bourque & Fourqurean, 2013; W. Kenworthy et al., 2018; Tuya et al., 2017). However, there are numerous techniques such as protective cages, or biodegradable lattices, artificial seagrass, in ground fertilizer application, and sediment tubes that aim to minimize environmental disturbances and which can be successfully integrated into restoration projects utilizing fertilizers (Hall et al., 2006; Hammerstrom et al., 1998; W. J. Kenworthy et al., 2018; Li et al., 2019; MacDonnell et al., 2022; Temmink et al., 2020; Tuya et al., 2017).

Multiple field and mesocosm seagrass studies investigating the use of Osmocote^{TM} have yielded generally similar results (Peralta et al., 2003; Pereda-Briones et al., 2018; Tanner & Parham, 2010). Both struvite/Osmocote^{TM} experiments could be considered extensions of these previous investigations with real world applications. However, it must be noted that a successful mesocosm scale study such as this one cannot simply be scaled up to field applications. Rather, it would require the additional understanding of local environmental conditions and applied restoration techniques that enhance the success rate. Therefore, a future field study would be recommended to optimize the dose of struvite in different biogeochemical conditions and assess associated operational efforts and costs.

4.3. Implications/Applications of Struvite

The integration of struvite in restoration projects could have multiple advantages for both environmental management and sustainability of wastewater treatment. First, more research is needed, but struvite is potentially less harmful for the environment than traditional commercial fertilizers. For example, struvite is sourced from wastewater, a source of eutrophication for many coastal systems (Mayer et al., 2016).

The N content of struvite is also relatively low, and while it still provides plants with nutrients, it limits excess fertilization and resulting nitrous oxide emissions (Rahman et al., 2014). Second, that struvite is sustainable and locally sourced has global implications as P resources are being depleted in an accelerating rate, and there are indications that demand will surpass supply within the next 20 years (Nedelciu et al., 2020). The processing of struvite allows for the production of a P fertilizer without dealing with the instability and increasing costs of importing fertilizer (Rufí-Salís et al., 2020; Ye et al., 2020). Finally, the feasibility of using struvite on multiple scales has been demonstrated in experiments and industrial applications, indicating a practical and readily available treatment process (Ghosh et al., 2019).

The advantages of struvite in reducing pollution and phosphate shortages, combined with its feasibility, make it an attractive alternative P and N fertilizer. Struvite is a recognized slow-release terrestrial nutrient amendment with a low environmental footprint. However, struvite application is still limited due to its high price in comparison to conventional mineral fertilizers and availability. Therefore, extending application of struvite into areas such as restoration could potentially create a new market, thus making struvite more affordable and available. This is particularly important since struvite represents a very important aspect of circular economy in water management.

5. Summary and Conclusions

Because of the current need for effective fertilization methods that minimize environmental risk, this study evaluated the wastewater by-product struvite and its potential to enhance seagrass growth under simulated natural conditions. Seagrass growth metrics (shoots, length, biomass) in plots fertilized with struvite were consistently equal to or better than the commercial fertilizer Osmocote^{TM}. This improvement in seagrass performance was provided while also producing lower porewater nutrient release from equal P fertilization doses, likely due to the slower release of nutrients from struvite delivering a low but sustained load of N and P to the rhizosphere. Excessive N inputs from the Osmocote^{TM} treatment in the first experiment may have even reduced performance of treated plots compared to the unfertilized control. Measurements of porewater nutrients and visual observations indicated that struvite has a lower solubility and is therefore longer lasting compared to Osmocote^{TM} in marine conditions. Other possible factors in plant performance, including the effects of specific nutrients (i.e. temporal delays in N/P tissue concentration, micronutrient differences), current flow (possibly increasing nutrient diffusion), and sediment particle size (affecting dissolution rates and redox potential), will require further investigation.

Future studies should apply the results of this experiment in multiple coastal systems, ensuring that results are not constrained to a seagrass mesocosm setting. Testing the solubility of struvite in different environments may reveal more applications for the fertilizer. Experiments should include other seagrass species with diverse nutrient requirements, and ideally, a restoration experiment would take place over multiple growing seasons to determine how long struvite remains effective. Special consideration should also be given toward testing the effectiveness of struvite in a more N-limited environment, where other fertilizers may have a better advantage. This study was a first ever attempt to apply struvite in marine restoration project, serving as an example of interdisciplinary merger between wastewater treatment engineering and restoration ecology. The positive results here should encourage future research and field activities to further explore the application of struvite and similar materials for restoration projects.

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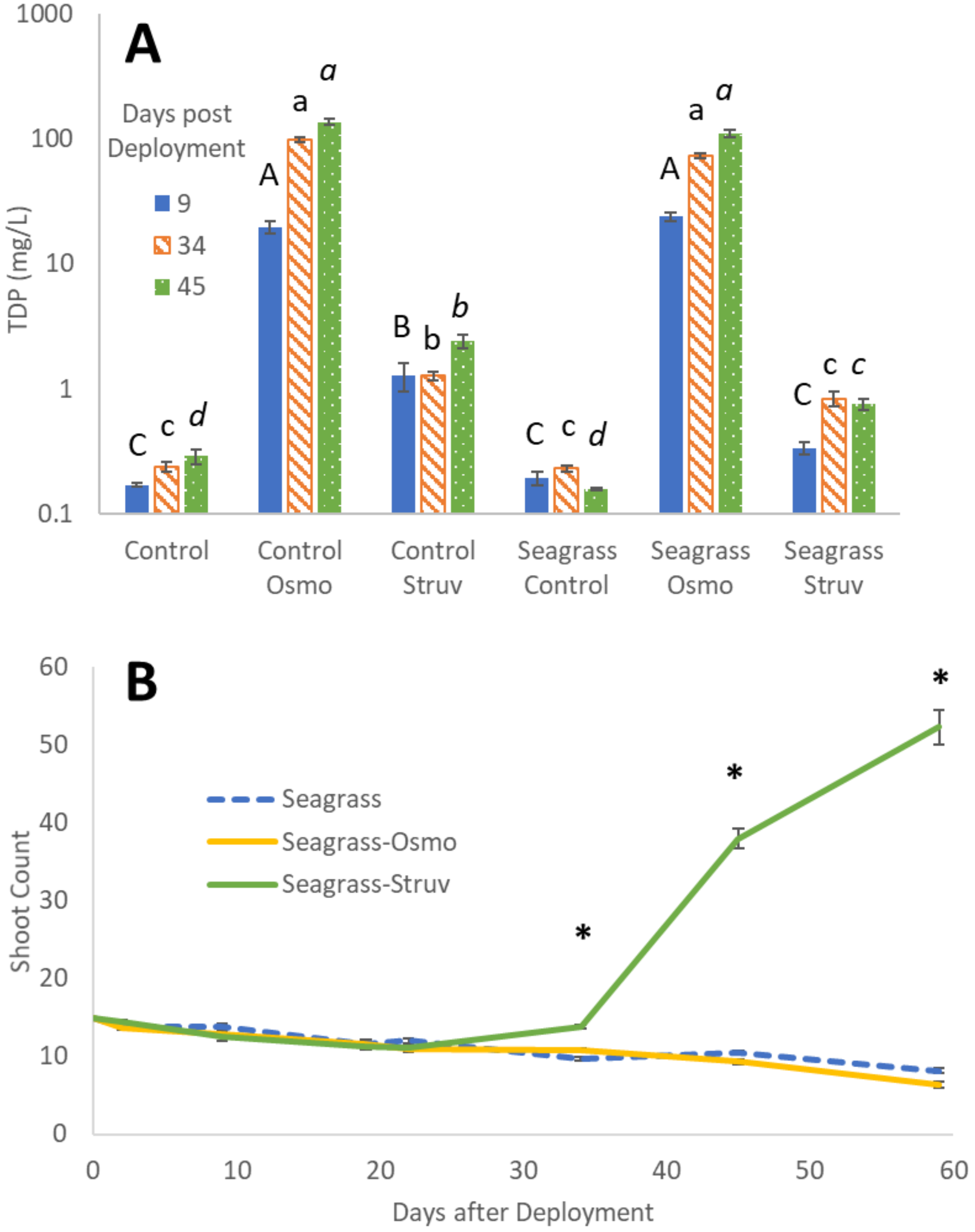

Figure 1. Porewater total dissolved phosphorus (TDP) (A), and seagrass shoot count (B) taken during the first mesocosm experiment. The treatments were labelled Seagrass (for the unfertilized seagrass plots), Seagrass-Osmocote^{TM} (for planted plots fertilized with Osmocote^{TM}), and Seagrass-Struv (planted plots fertilized with struvite). The asterisks designate significant differences between treatments for the same sample dates. Points represent the mean of six replicates (± SE).

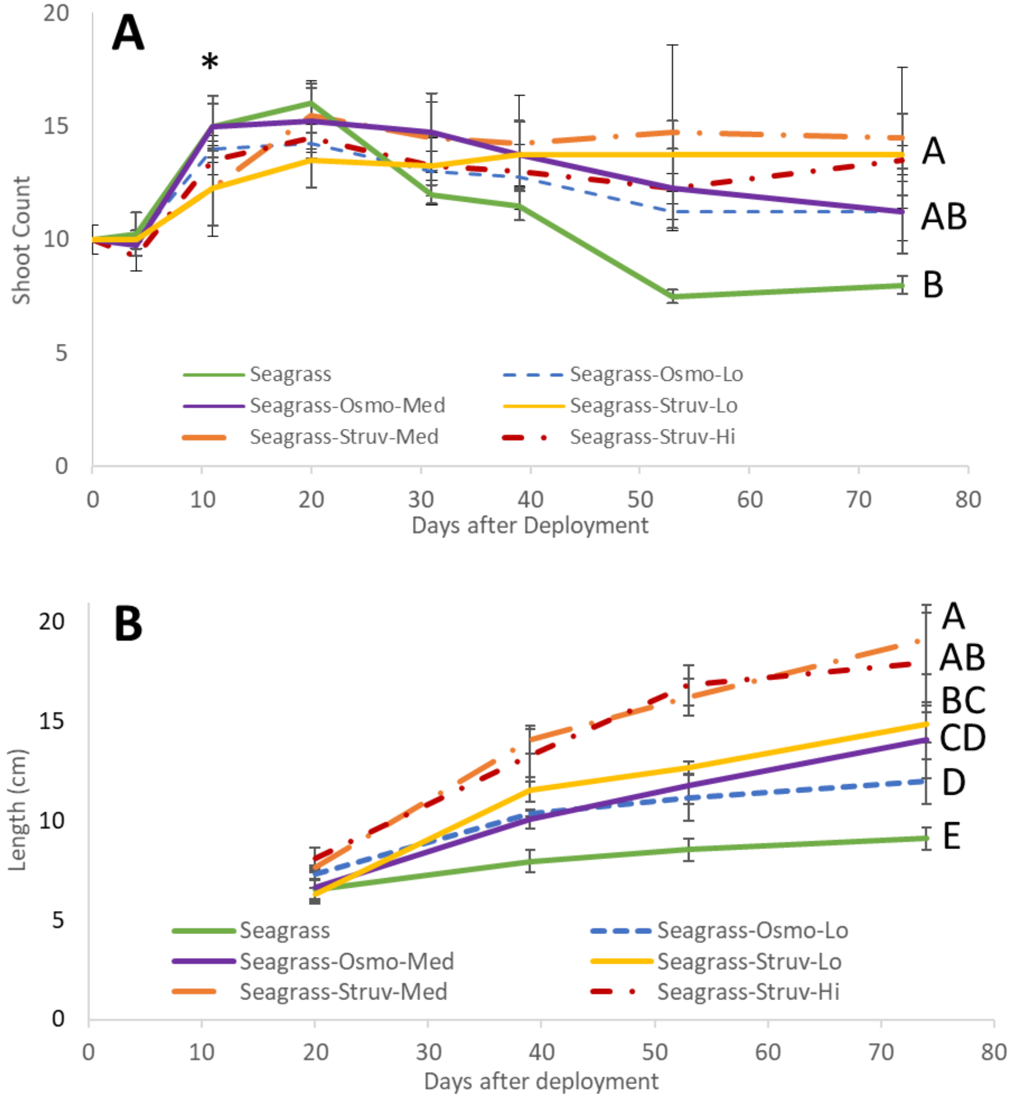

Figure 2. Shoot count (A) and blade length (B) from the second mesocosm experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote^{TM}), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). *: Five shoots were added to each plot to match the first/single dose experiment. Letters designate significant differences between treatments for the same sample dates. Points represent the mean of four replicates (± SE), except for above-ground biomass, which had two to four replicates.

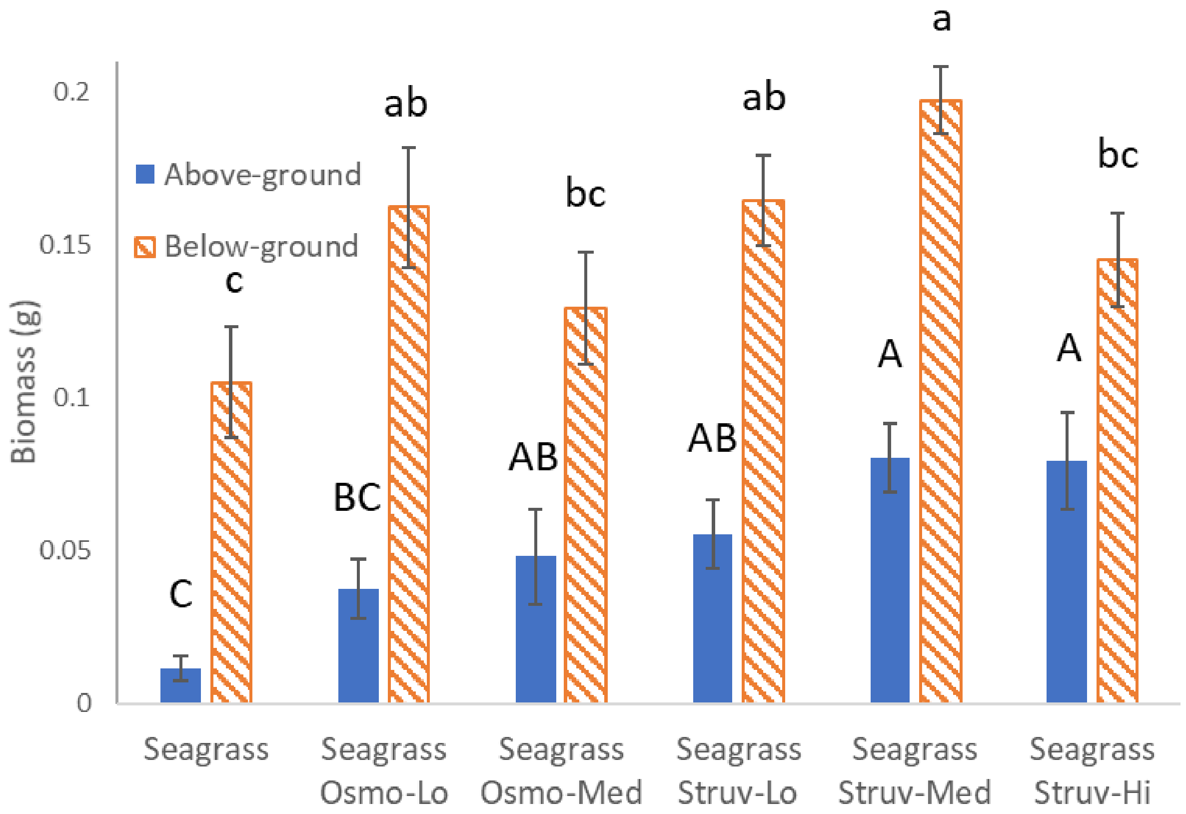

Figure 3. Above and belowground biomass from the second mesocosm experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Letters designate significant differences between treatments for the same sample dates. Points represent the mean of four replicates (± SE), except for above-ground biomass, which had two to four replicates.

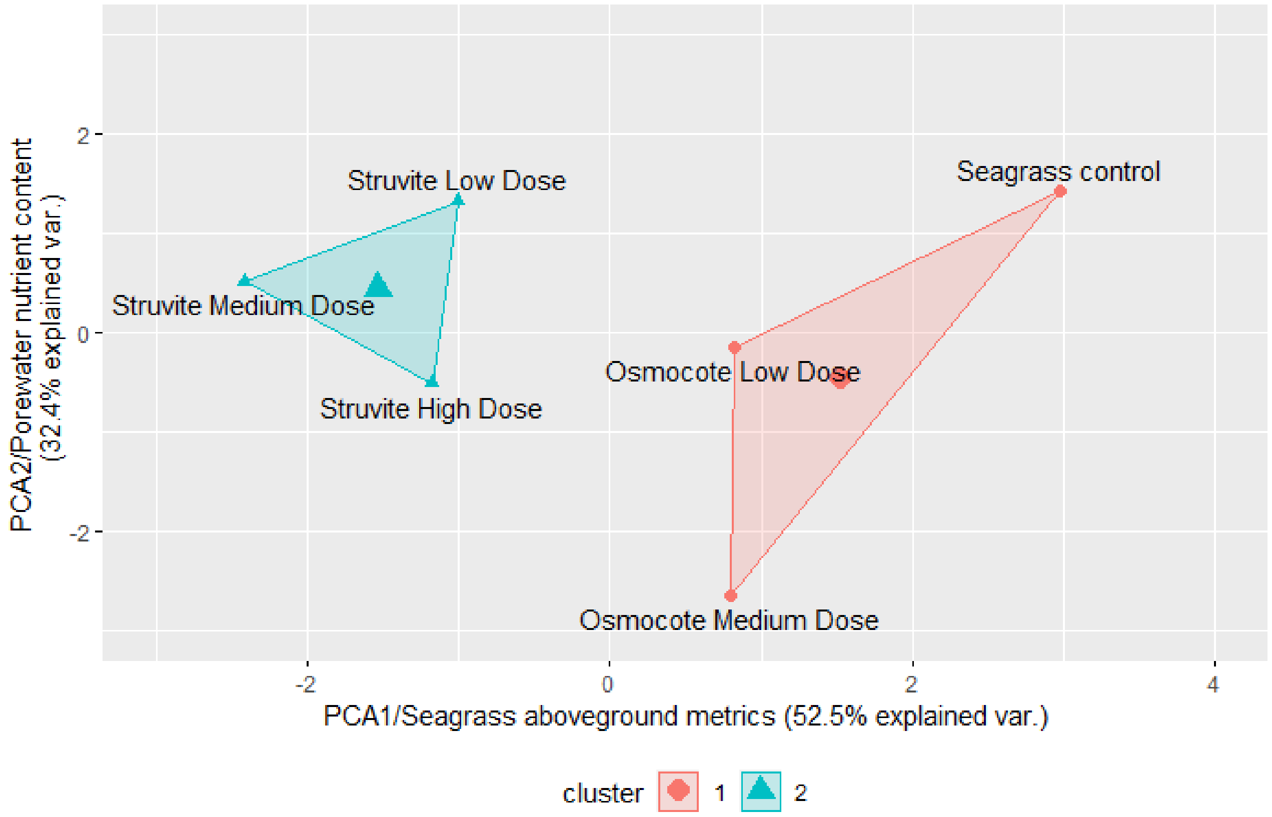

Figure 4. Clustering results of treatment methods from the second experiment. No overlapping clusters were formed, indicating a significantly different global effect of struvite onto the water chemistry and plant growth characteristics compared to Osmocote^{TM} treatment or control plot. Seagrass aboveground metrics (shoot count, blade length and aboveground biomass) were heavily corelated ($r > 95\%$, $p < 0.001$) with first principal component which explained 52.5% of the variance in the dataset. Porewater nutrient dynamics such as TDN and TDP were most corelated ($p < 0.05$) and contributing to second principal component which explained 32.4% of the variance in the dataset. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote^{TM}), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite).

Table S-1. Two-way linear mixed effects test results for shoot count and TDP from the single dose experiment/Experiment #1. Factors include treatment, date sampled, and the interaction between these factors.

	Variable					
	Shoot			TDP		
Source	----------count----------			----------mg L^{-1}----------		
Parameter	DF	F statistic	P value	DF	F statistic	P value
Treatment	2	35.91	< 0.0001	5	246.7	< 0.0001
Date	7	10.07	< 0.0001	2	19.50	< 0.0001
Treatment x Date	14	27.95	< 0.0001	10	2.201	0.0328

Table S-2. Two-way linear mixed effects test results for seagrass metrics and porewater nutrients from the multi-dose experiment/Experiment #2. Factors include treatment, date sampled, and the interaction between these factors.

	Variable											
	Shoot			Length			TDN			TDP		
Source	----------count----------			----------cm----------			----------mg L^{-1}----------					
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	5	0.5703	0.7219	5	13.57	< 0.0001	8	22.37	< 0.0001	8	42.30	< 0.0001
Date	6	18.83	< 0.0001	3	83.75	< 0.0001	2	1.820	0.1686	3	128.2	< 0.0001
Type x Date	30	1.683	0.0289	15	2.118	0.0265	16	1.106	0.3636	24	6.341	< 0.0001

Table S-3. Linear mixed effects test table for biomass taken at the end of the multi-dose experiment/Experiment #2.

	Variable					
	Aboveground Biomass			Belowground Biomass		
Source	----------g----------					
Parameter	DF	F statistic	P value	DF	F statistic	P value
Type	4	5.231	0.0077	4	4.560	0.0131

Table S-4. Linear mixed effects test table for tissue nutrients (percent weight) taken at the end of the multi-dose experiment/Experiment #2.

	Variable											
	Aboveground %TN			Aboveground %TP			Belowground %TN			Belowground %TP		
Source	----------Percent----------											
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	4	0.784	0.560	3	0.583	0.639	4	3.072	0.049	4	0.539	0.709

Table S-5. Linear mixed effects test table for tissue nutrient mass (biomass x % nutrients) taken at the end of the multi-dose experiment/Experiment #2. Combined weights present the aboveground TN and TP weights were removed for the analysis (n= 2 for Osmocote^{TM} treatments, unfertilized seagrass removed). Belowground weights for both TN and TP did not have combined samples.

	Variable											
	Aboveground TN Mass			Aboveground TP Mass			Belowground TN Mass			Belowground TP Mass		
Source	----------g----------											
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	3	1.619	0.2370	3	2.524	0.1070	4	3.276	0.0406	4	3.705	0.0273

Table S-6. Mean sediment TC/TN values taken at the end of the multi-dose experiment/Experiment #2. The treatments were labelled control (unfertilized, unplanted plots), Osmo (unplanted fertilized with Osmocote), Struv (unplanted fertilized with struvite), S-Control (unfertilized seagrass plots), S-Osmo-Lo and S-Osmo-Med (planted plots fertilized with Osmocote), S-Struv-Lo, S-Struv-Med, and S-Struv-Hi (planted plots fertilized with struvite).

	Variable			
	TC		TN	
	Mean	SE	Mean	SE
Treatment	----------mg/kg----------			
Control	52.55^{NS}	2.22	2.058^{NS}	0.009
Control-Osmo	57.55^{NS}	2.24	2.073^{NS}	0.015
Control-Struv	53.53^{NS}	6.73	2.036^{NS}	0.016
Seagrass	52.09^{NS}	4.33	2.020^{NS}	0.032
Seagrass-Osmo-Lo	50.56^{NS}	3.28	2.0259^{NS}	0.005
Seagrass-Osmo-Med	49.46^{NS}	3.56	2.053^{NS}	0.027
Seagrass-Struv-Lo	52.02^{NS}	6.17	2.057^{NS}	0.027
Seagrass-Struv-Med	48.70^{NS}	5.02	2.023^{NS}	0.026
Seagrass-Struv-Hi	58.16^{NS}	5.63	2.096^{NS}	0.02

Table S-7. Mixed effects test table for sediment TC/TN taken at the end of the multi-dose experiment/Experiment #2.

Variable						
	TC			TN		
Source	---mg/kg---					
Parameter	DF	F statistic	P value	DF	F statistic	P value
Treatment	8	0.5028	0.8435	8	1.376	0.2514

Table S-8. Mean porewater nutrient measurements from the second experiment. The treatments were labelled Control (unfertilized, unplanted plots), Control-Osmo (unplanted fertilized with Osmocote^{TM}), Control-Struv (unplanted fertilized with struvite), Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote^{TM}), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Only TDP was analyzed for Day 31, therefore TDN values at that date are designated "NA." Letters within biomass type represent a significantly different mean based on linear mixed model analyses (NS= not significant).

Variable					
		TDN		TDP	
		Mean	SE	Mean	SE
Days after Deployment	Treatment	----------mg/L----------			
6	Control	1.51^{NS}	0.38	0.128^{D}	0.004
	Control-Osmo	12.03^{NS}	5.16	9.640^{AB}	3.685
	Control-Struv	4.35^{NS}	1.52	2.093^{C}	0.276
	Seagrass	1.41^{NS}	0.45	0.182^{D}	0.021
	Seagrass-Osmo-Lo	7.89^{NS}	4.34	4.750^{BC}	1.169
	Seagrass-Osmo-Med	26.8^{NS}	7.53	17.68^{A}	6.738
	Seagrass-Struv-Lo	4.33^{NS}	1.60	2.795^{C}	0.962
	Seagrass-Struv-Med	3.32^{NS}	0.88	1.943^{C}	0.211
	Seagrass-Struv-Hi	4.79^{NS}	0.86	2.620^{C}	0.351
20	Control	1.10^{NS}	0.16	0.133^{C}	0.010
	Control-Osmo	19.8^{NS}	2.14	0.508^{A}	0.102
	Control-Struv	5.59^{NS}	1.91	0.303^{ABC}	0.138
	Seagrass	0.78^{NS}	0.14	0.134^{C}	0.016
	Seagrass-Osmo-Lo	6.20^{NS}	2.33	0.289^{ABC}	0.094
	Seagrass-Osmo-Med	12.02^{NS}	0.82	0.472^{AB}	0.279
	Seagrass-Struv-Lo	2.47^{NS}	0.42	0.272^{ABC}	0.107
	Seagrass-Struv-Med	2.58^{NS}	0.80	0.187^{BC}	0.021
	Seagrass-Struv-Hi	6.81^{NS}	1.73	0.322^{ABC}	0.113

Table S-8. Continued.

Variable					
		TDN		TDP	
		Mean	SE	Mean	SE
Days after Deployment	Treatment	----------mg/L----------			
31	Control	NA	NA	0.110^{D}	0.009
	Control-Osmo	NA	NA	2.455^{BC}	0.560
	Control-Struv	NA	NA	2.923^{ABC}	0.966
	Seagrass	NA	NA	0.114^{D}	0.016
	Seagrass-Osmo-Lo	NA	NA	1.47^{C}	0.375
	Seagrass-Osmo-Med	NA	NA	9.733^{AB}	6.667
	Seagrass-Struv-Lo	NA	NA	0.163^{D}	0.038
	Seagrass-Struv-Med	NA	NA	1.353^{C}	0.166
74	Control	1.97^{NS}	0.19	0.085^{C}	0.007
	Control-Osmo	12.2^{NS}	7.54	0.488^{A}	0.135
	Control-Struv	7.75^{NS}	2.00	0.159^{BC}	0.054
	Seagrass	1.68^{NS}	0.15	0.084^{C}	0.021
	Seagrass-Osmo-Lo	5.39^{NS}	0.54	0.261^{AB}	0.067
	Seagrass-Osmo-Med	17.3^{NS}	4.98	0.551^{A}	0.105
	Seagrass-Struv-Lo	4.04^{NS}	1.23	0.162^{BC}	0.064
	Seagrass-Struv-Med	4.74^{NS}	1.03	0.143^{BC}	0.023
	Seagrass-Struv-Hi	9.32^{NS}	1.62	0.156^{BC}	0.017

Table S-9. Mean percent tissue nutrient content and ratio taken at the end of the second experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote^{TM}), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Combined weights present the aboveground TN and TP weights were removed for the analysis (n= 2 for Osmocote^{TM} treatments, unfertilized seagrass removed). Belowground weights for both TN and TP did not have combined samples. Letters within biomass type represent a significantly different mean based on linear mixed model analyses (NS= not significant).

		TN		TP		TN:TP	
		Mean	SE	Mean	SE	Mean	SE
Biomass Type	Treatment	----------Percent----------				Wt/Wt Ratio	
Above-ground	Seagrass	1.90^{NS}	NA	NA	NA	NA	NA
	Seagrass-Osmo-Lo	2.14^{NS}	0.11	0.258^{NS}	0.016	8.33^{NS}	0.57
	Seagrass-Osmo-Med	2.08^{NS}	0.11	0.249^{NS}	0.017	8.47^{NS}	0.88
	Seagrass-Struv-Lo	2.34^{NS}	0.23	0.236^{NS}	0.007	10.01^{NS}	1.20
	Seagrass-Struv-Med	2.31^{NS}	0.14	0.246^{NS}	0.010	9.38^{NS}	0.29
	Seagrass-Struv-Hi	NA	NA	NA	NA	NA	NA
Below-ground	Seagrass	0.65^{AB}	0.06	0.166^{NS}	0.018	3.90^{NS}	0.36
	Seagrass-Osmo-Lo	0.53^{B}	0.07	0.154^{NS}	0.009	3.44^{NS}	0.47
	Seagrass-Osmo-Med	0.84^{A}	0.06	0.179^{NS}	0.008	4.71^{NS}	0.36
	Seagrass-Struv-Lo	0.66^{AB}	0.05	0.168^{NS}	0.011	3.94^{NS}	0.29
	Seagrass-Struv-Med	0.71^{AB}	0.07	0.164^{NS}	0.011	4.32^{NS}	0.45
	Seagrass-Struv-Hi	NA	NA	NA	NA	NA	NA

Figure S-1. Image showing an example of the differences in above and belowground biomass in seagrasses from the first experiment. Observable stunted roots (possibly root burn) are visible in the Osmocote$^{\mathrm{TM}}$ treated plots (SP) versus the control (S) and struvite treated plots (SS).

Declaration of Interest Statement

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author Contributions

MacDonnell, C.: Conceptualization, data curation, formal analysis, methodology, visualization, project administration, investigation, roles/writing- original draft/review and editing.

Bydalek, F.: Conceptualization, data curation, formal analysis, investigation, resources, methodology, visualization, roles/writing- original draft/review and editing.

Osborne, T.: Conceptualization, data curation, formal analysis, supervision, resources, methodology, visualization, roles/writing- review and editing.

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Makinia, J.: Resources, funding acquisition, writing- review and editing.

Inglett, P.W.: Conceptualization, supervision, data curation, project administration, formal analysis, methodology, visualization, roles/writing- original draft/review and editing.